EPA United States Environmental Protection Agency

Manual for the Certification of Laboratories Analyzing Drinking Water

Criteria and Procedures Quality Assurance

Fifth Edition

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US Environmental Protection Agency
Office of Water
Office of Ground Water and Drinking Water
Technical Support Center
Cincinnati, Ohio 45268

DISCLAIMER

The U.S. Environmental Protection Agency's (EPA's) Office of Ground Water and Drinking Water, in the Office of Water, and the Office of Research and Development of the U.S. Environmental Protection Agency prepared this manual. Those Offices as well as EPA's ten Regional Offices have reviewed this manual. EPA intends to use this manual for its own use in certifying laboratories for analysis of drinking water contaminants. In order to assume primary enforcement responsibility for the drinking water regulations, a State must either have available laboratory facilities, certified by the Administrator, capable of conducting analytical measurements of drinking water contaminants, or establish and maintain its own program for certification of laboratories. States wishing to adapt the procedures and criteria of this manual for their own certification program should revise it to accurately reflect accurately their State certification program.

This is a guidance manual and not a regulation. It does not change or substitute for any legal requirement. While EPA has made every effort to ensure the accuracy of the manual's discussion, the obligations of the regulated community are determined by the relevant statutes, regulations or other legally binding requirements. The manual obviously can only reflect the regulations in place at this time of its preparation. Consequently, for any definitive description of current legal obligations, the public should not rely only on the discussion in the manual. This manual is not a rule, is not legally enforceable, and does not confer legal rights or impose legal requirements upon any member of the public, States or any other Federal agency. In the event of a conflict between the discussions in this manual and any statute or regulation, this document is not controlling. The word "should" in this manual does not connote a requirement but does indicate EPA's strongly preferred approach to ensure the quality of laboratory results. EPA may decide to revise this manual without public notice to reflect changes to its approach or to clarify and update the text.

The mention of commercial products in this manual does not constitute an endorsement of the use of that product by EPA.

Acknowledgments

This edition of the manual was prepared through the efforts of many individuals, including representatives from the U.S. Environmental Protection Agency's Office of Ground Water and Drinking Water (OGWDW), Office of Research and Development (ORD), Regional Offices and the States. It has as its foundation previous editions of the manual. Contributors to the previous editions of the manual are listed in EPA documents EPA 815-B-97-001 March 1997, EPA/570/9-90/008 April 1990, EPA-570/9-82-002 October 1982 and EPA 600/8-78-008 May 1978. Contributors to this edition are listed below.

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Preface

Since 1978, the U. S. Environmental Protection Agency (EPA) has implemented a certification program for laboratories performing drinking water analyses for compliance with regulations issued pursuant to the Safe Drinking Water Act. These laboratories include EPA Regional laboratories, certain Federal laboratories, Tribal Nation laboratories, principal State laboratories in primacy States, and drinking water laboratories in non-primacy States. This manual describes criteria and procedures that EPA uses in evaluating laboratories for certification. EPA has concluded that laboratories that adopt the approaches discussed in this manual will generate reliable analytical data. Consequently, EPA recommends that States follow these procedures and criteria in their certification decisions.

This document is the fifth edition of the manual, describing the program's implementation procedures and technical criteria. It supersedes the Manual for the Certification of Laboratories Analyzing Drinking Water, EPA-815-B-97-001 (March 1997).

The manual was revised to address: 1)the recently promulgated drinking water regulations and methods; and 2) Agency policy (Office of Ground Water and Drinking water memo, October 1, 2002)(see Appendix F) that, at the discretion of each state's Certification Authority (CA), allows National Environmental Laboratory Accreditation Program (NELAP) accreditation to be accepted in lieu of drinking water certification in terms of producing data for compliance monitoring purposes.

A committee chaired by the EPA's Office of Ground Water and Drinking Water (OGWDW) with participation of the National Exposure Research Laboratories in Cincinnati (NERL-Ci) and representatives from the EPA Regions and the States prepared this document. Its goal is to improve implementation of the SDWA in light of newly approved methodology and additional experience with the program.

Like previous editions, this edition is in loose-leaf format which will allow the EPA to update it more easily. Holders of this manual should check with the EPA Region or the State Certification Officers to make sure their manual is current. Additional copies of this manual may be obtained from the EPA, OGWDW, 26 W M.L. King Dr., Cincinnati, OH, 45268, fax number 513 569-7191, or by calling the Safe Drinking Water Hotline at 800 426-4791. The manual is also posted on the Internet at www.epa.gov/safewater/certlab/labindex.html.

To ensure uniformity in its program in all the Regions, EPA uses the certification criteria in this manual for evaluating all drinking water laboratories that it certifies. The Agency also uses this manual to determine the adequacy of State certification programs for drinking water laboratories. States are encouraged to use the criteria in this manual to evaluate all laboratories that they certify.

Generally the term "must" in this manual refers to elements that are required by the National Primary Drinking Water Regulations or the approved drinking water methods. This manual uses the term "should" to describe criteria and procedures that in OW's judgement are necessary for laboratories to produce data that are scientifically valid and defensible, and are of known and acceptable precision and accuracy.

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Chapter I
Introduction

Public water systems serving at least 25 persons or having at least 15 service connections must comply with the Safe Drinking Water Act (SDWA) and the requirements of the National Primary Drinking Water Regulations (NPDWR) (40 CFR 141). Section 1401(1)(D) of the Act defines a National Primary Drinking Water Regulation to include "criteria and procedures . . . [for] quality control and testing procedures to insure compliance . . . " EPA regulations require community water systems to conduct monitoring for compliance with the drinking water standards. In addition, regulations provide that analyses must be conducted by laboratories certified by EPA or the States. The regulations also established requirements for laboratory certification..

The regulations governing primacy at 40 CFR 142.10(b)(4) require, as a condition of primary enforcement responsibility (primacy), that a state have laboratory facilities available (the Principal State Laboratory) certified by the regional administrator. In addition, the regulations governing certification (40 CFR 141.28) require that all testing for compliance purposes be performed by certified laboratories except that turbidity, free chlorine residual, temperature, pH, alkalinity, calcium, conductivity, orthophosphate, TOC, SUVA, daily chlorite, and silica may be performed by anyone acceptable to the State. This manual is intended to assist EPA in implementing 40 CFR 142.10(b)(4) by specifying criteria and procedures EPA uses in evaluating principal State laboratories for certification. States with primacy may also choose to use equivalent criteria and procedures similar to those in this manual for their own certification programs.

To obtain and maintain primacy, a State must comply with 40 CFR 142.10, which includes the following provisions:

> The establishment and maintenance of a State program for the certification of laboratories conducting analytical measurements of drinking water contaminants pursuant to the requirements of the State primary drinking water regulations including the designation by the State of a laboratory officer, or officers, certified by the Administrator, as the official(s) responsible for the State's certification program. The requirements of this paragraph may be waived by the Administrator for any State where all analytical measurements required by the State's primary drinking water regulations are conducted at laboratories operated by the State and certified by the Agency. (40 CFR 142.10(b)(3)(i))
>
> Assurance of the availability to the State of laboratory facilities certified by the Administrator and capable of performing analytical measurements of all contaminants specified in the State primary drinking water regulations . . . (40 CFR 142.10(b)(4)).

NOTE: Reference to the Administrator of EPA also refers to his or her designee.

The requirement for a laboratory certification program extends to EPA Regional laboratories, Tribal Nation laboratories, principal State laboratories in primacy States, and laboratories that perform analyses under the Safe Drinking Water Act in States without primacy. If all required analyses are not performed in principal State laboratories, primacy States must have a certification program for certifying other drinking water laboratories (40 CFR 142.10(b)(3)(i)).

EPA's Technical Support Center (TSC) in Cincinnati, Ohio, with the assistance of the National Exposure Research Laboratory in Cincinnati, Ohio (NERL-Ci), is responsible for determining the certification status for EPA's Regional laboratories in microbiology, chemistry, and radiochemistry. Regional certification officers are responsible for the certification of the principal State laboratory in each primacy State, and are also responsible for certifying all Tribal Nation laboratories and laboratories in non-primacy States. Primacy States with certification programs are responsible for certifying the other drinking water laboratories in their State, (i.e., laboratories other than the principal State Laboratory).

Regional Laboratories must successfully analyze a set of proficiency testing samples (PTs) at least annually for all regulated contaminants for which they wish to be certified, by each method for which they wish to be certified (40 CFR

141.23 and 141.24), and pass an on-site evaluation at least every three years. An on-site evaluation determines conformance with the criteria specified in this manual. Principal State laboratories must (40 CFR 141.23 and 141.24) successfully analyze a complete set of proficiency testing (PT) samples from a source acceptable to the Region at least annually for the contaminants and methods included in the regulations which the State has adopted, and pass an on-site evaluation every three years. EPA auditors use the criteria in this manual for the on-site audits of the Regional and principal State laboratories.

Chapter II describes the responsibilities of each of the parties involved in the certification program. Chapter III describes how the program operates. Chapters IV, V, and VI cover the technical criteria to be used during the on-site evaluation of a laboratory for chemistry, microbiology, and radiochemistry, respectively. Optional audit forms are also included in Chapters IV, V, and VI. The appendices include the following: a recommended protocol and format for conducting on-site laboratory evaluations which may be used by the laboratory auditors; frequently used abbreviations and definitions; EPA's policy on third-party auditors; EPA's policy on National Environmental Laboratory Accreditation Program (NELAP) accreditation as an alternate for drinking water certification; a list of contaminants a principal State laboratory must (primacy citation 142.10(b)(4)) have the capability to analyze, analytical methods for microbiology analyses; and recommended chain-of-custody procedures to be used when necessary.

Chapter II
Responsibilities

The success of the laboratory certification program depends upon cooperation among the organizations responsible for its implementation. Within the Agency, responsibilities for laboratory certification are shared by the Office of Ground Water and Drinking Water (OGWDW), the Office of Research and Development (ORD), and the Regional Offices.

Office of Ground Water and Drinking Water (OGWDW) and Office of Research and Development (ORD)

OGWDW, with assistance from ORD, has the responsibility for developing and implementing the national certification program for laboratories that analyze drinking water samples and for implementing the Safe Drinking Water Act. These responsibilities include the following:

- Propose and promulgate regulations;
- Assess national laboratory capacity and capability;
- Review the EPA Regional certification programs annually and evaluate the resources and personnel available in each EPA Region to carry out the certification program;
- Develop guidance and respond to questions and comments;
- Develop technical and administrative certification criteria to support future regulations;
- Revise this manual when necessary;
- Conduct triennial on-site audits of each Regional laboratory for chemistry, microbiology, and radiochemistry
- Maintain a database of laboratory ID Codes
- Develop and participate in training courses to support the certification program;
- Provide technical assistance to EPA and the States;
- Develop and evaluate methods for the analysis of drinking water contaminants.

EPA Regions

The Regions oversee the certification programs in the States and Tribal Nations. The Regions' responsibilities are to:

- Determine the certification status for the principal State/Tribal laboratory system in each primacy State/Tribe;
- Perform an annual review of State/Tribal certification programs and proficiency testing results and monitor the adequacy of State/Tribal programs for certifying laboratories, as described in Chapter III;
- Sponsor annual meetings for the state Certification Officers (COs);
- Provide technical assistance to the States' EPA-certified drinking water laboratories, as needed;
- Manage the certification program for drinking water laboratories in non-primacy States using the criteria in this manual.
- Manage the certification program for drinking water laboratories on Tribal Nation lands using the criteria in this manual.

This last duty may be performed by the State, but the Region retains responsibility for the on-site evaluation of the designated Tribal Nation principal laboratory. Drinking water laboratories may be evaluated by the Region, or under a Region-approved program carried out by a designated State program. In either case, this manual is the basis for the on-site audits, conducted by EPA, of principal State laboratories, laboratories on Tribal Nation lands, and drinking water laboratories in non-primacy States.

The Regional laboratory should maintain certification for as many regulated contaminants as its resources permit. This enhances both EPA's technical assistance capabilities and credibility with those it certifies. It also ensures the laboratory capability to analyze samples for possible enforcement actions and for States which do not have primacy. Reciprocal agreements with other regions to share scarce resources are recommended.

Primacy States

Primacy States, in which all drinking water compliance samples are **not** analyzed at State operated laboratories, are required to establish and maintain a State program for the certification of laboratories conducting analyses of drinking water compliance samples [see 40 CFR 142.10(b)(3)(i)]. EPA encourages the States to base certification of drinking water laboratories either upon criteria contained in this manual or upon state-developed equivalents that are at least as stringent as this manual. Primacy States must establish and maintain a state program for the certification of laboratories conducting analytical measurements of drinking water contaminants pursuant to the requirements of the State primary drinking water regulations. The States must designate a "laboratory officer or officers, certified by the Administrator or designee as the official(s) responsible for the certification program." (40 CFR §142.10 (a)(3)(i))

States are responsible for the certification of the public and private laboratories in their State. This includes auditing the laboratories and reviewing the PT data. States should also provide technical assistance to laboratories. They may also choose to certify laboratories outside their State either by an on-site evaluation or reciprocity.

Chapter III
Implementation

1. Evaluation of Certification Programs

OGWDW and the Regions monitor the certification programs under their purview annually. These offices assess the adequacy of programs for certifying laboratories by evaluating each program's scope, staffing, resources, policies, procedures, and effectiveness. This should be done in person during an on-site audit when possible, and at least by means of a questionnaire in the other years. The adequacy of these essential program elements is evaluated by:

- Reviewing the program plan, responsibilities, organizational structure, staff (including educational background and experience), scope and description of the certification process, downgrading criteria and processes, and use of PT samples;
- Requesting an annual program report that includes program highlights, training, continuing education efforts, number of on-site evaluations performed, listing of laboratories certified by discipline or contaminant, and any certification downgrading or upgrading actions along with reasons for those actions;
- Observing the state certification officers on-site audits of drinking water laboratories;
- Encouraging State and Regional laboratory auditors to observe on-site audits of their own and other laboratories as on-the-job training;
- Sponsoring annual meetings of certification officers to discuss program issues, policies, and problems. Key Regional, NERL, OGWDW, and State personnel should be invited to participate.

2. Requirements for Certification of Laboratories

In order to be eligible to analyze compliance samples under the Safe Drinking Water Act, Regional and Principal State laboratories should meet the minimum criteria specified in this manual, which includes passing an on-site audit at least once every three years, and satisfactorily analyzing a set of PT samples annually.

The Office of Ground Water and Drinking Water (OGWDW) will accept NELAP accreditation as equivalent to Drinking Water certification, if all requirements of the drinking water program are met.

3. Individual(s) Responsible for the Certification Program

The Technical Support Center, with the assistance from NERL-Ci is responsible for certifying the regional laboratories; the Regions are responsible for certifying their States' principal laboratory systems and Tribal Nations' laboratories; and the States are responsible for certifying private, municipal, non-principal state, and governments laboratories.

The certification program personnel in each Region should consist of a certification authority(s) (CA), the certification program manager, and a certification team comprised of certification officers (COs) and technical experts. Additional third party auditors and experts may be used. However, third parties has have no authority for certification decisions. Third party auditing is discussed in Section 4.2.

The **Certification Authority (CA)** is the person who has signature authority for all certification decisions. This is the Chief of the Technical Support Center and the Regional Administrator in the Regions. The RA may delegate this authority to a lower level.

The **Certification Program Manager** (CPM) is the person responsible for managing the drinking water laboratory certification activities in the Region.

The **Certification Officers (COs)** are the regional and state personnel who have the responsibility of certifying laboratories under their purview. 40 CFR 142.10(b)(3)(i) requires Primacy States to designate a person certified by the Administrator as the official responsible for the State's certification program. This person would be the State certification authority.

4. On-Site Laboratory Audit

4.1 On-Site Laboratory Audit Team

The certification program manager should establish one or more teams of certification officers and auditors to audit laboratories. It is the responsibility of these teams to perform the on-site laboratory audits, review the laboratory PT data, and make recommendations to the CA concerning the certification status of the laboratories.

Team members should be experienced professionals, hold at least a bachelor's degree or equivalent education/experience in the discipline (chemistry, radiochemistry, microbiology or a related field) for which they certify, and have recent laboratory experience.

Team members should also have experience in laboratory evaluation and quality assurance, be familiar with the drinking water regulations and data reduction and reporting techniques, be technically conversant with the analytical techniques being evaluated, and be able to communicate effectively, both orally and in writing.

The on-site team should include at least one person knowledgeable in each area being audited (e.g., inorganic and organic chemistry, radiochemistry, and microbiology). COs need to successfully complete the appropriate EPA laboratory certification course. In addition, there should be a mechanism for COs to receive periodic training regarding newly promulgated regulations, newly adopted certification criteria, and new methods. This could be done by auditing the EPA CO Training Course and/or attending the required annual Regional and State CO Meetings.

4.2 Third Party Auditors

Certification programs may employ third party auditors who meet all of the qualifications listed above. In areas where experience does not exist within the certification team (e.g., asbestos), outside expert assistance may be obtained in the needed areas to assist the on-site team. Outside experts who have not attended the EPA certification officer training should be accompanied by a certification officer. Although these third parties may be used to assist EPA or State certification officers, they have no authority for certification decisions and they may not make final certification decisions. These decisions rest with the EPA or the State.

When using third party experts, it is critical to avoid conflicts of interest. A third party auditor who in any way stands to benefit by the certification status of the laboratory audited may not be used.

5. Plans for Certification of Laboratories

The certification program manager should develop plans for certifying drinking water laboratories under her/his authority. Written plans should include the following:

- Documentation of certification authority and certification officers and their education/experience;
- Schedules of laboratories to be audited;
- Specific types of analyses to be examined;
- Protocol to be followed;
- Strategy for assessing laboratory performance (e.g., PTs, data audits, etc.);
- Plans for providing technical assistance to laboratories which need upgrading.

6. Principal State Laboratories

To receive and retain primacy, the State must (40 CFR 142.10(b)(4)) have available laboratory facilities capable of performing analytical measurements for all the federally mandated contaminants specified in the State Primary Drinking Water Regulations. This laboratory or laboratories are considered the Principal State Laboratory System and must be certified by EPA.

7. Certification Process

The certification process begins when the laboratory director makes a formal request in writing to the certification authority to be certified. This application may be one of the following:

- A request for first-time certification for microbiology, chemistry, or radiochemistry;
- A request for certification to analyze additional or newly regulated contaminants;
- A request to reapply for certification after correction of deficiencies which resulted in the downgrading/revocation of certification status.

The response to a formal application for any of the above requests should be given within 30 days. At this time a mutually agreeable date and time should be set for the on-site laboratory audit.

Subsequent audits may be initiated by the CA or the laboratory.

A recommended protocol for conducting these audits is given in Appendix B.

Drinking water laboratories should specifically state that they plan to analyze drinking water samples when they request certification. If a laboratory has not been analyzing drinking water samples and does not plan to analyze drinking water samples, OGWDW, the Region or State may choose not to expend the resources to renew their certification.

8. Types of Certification

After review of PT sample results and an on-site visit, the certification authority should provide a written report within 45 days and classify the laboratory for each contaminant or group of contaminants according to the following rating scheme:

8.1 ***Certified*** - a laboratory that meets the regulatory performance criteria as explained in this manual and all other applicable regulatory requirements.

8.2 ***Provisionally Certified*** - a laboratory that has deficiencies but demonstrates its ability to consistently produce valid data within the acceptance limits specified in the NPDWR, and within the policy required by their certification authority. A provisionally certified laboratory may analyze drinking water samples for compliance purposes, if the said clients are notified of its downgraded status in writing, on any report. Provisional certification may not be given if the evaluation team believes that the laboratory cannot perform an analysis within the acceptance limits specified in the regulations.

8.3 ***Not Certified*** - a laboratory that possesses deficiencies and, in the opinion of the Certification Authority, cannot consistently produce valid data.

8.4 ***Interim Certification*** - interim certification may be granted in certain circumstances when it is impossible or unnecessary to perform an on-site audit. Interim certification status may be granted if, for example, the CA determines that the laboratory has the appropriate instrumentation, is using the approved methods, has adequately trained personnel to perform the analyses, and has satisfactorily analyzed PT samples, if available, for the contaminants in question. The CO should perform an on-site audit as soon as possible but no later than three years. An example of a situation where this type of certification is warranted would be a laboratory that has requested certification for the analysis of additional analytes that involve a method for which it already has certification. The CO should review the laboratory's quality control data before granting this type of certification.

9. Drinking Water Laboratories

For the purpose of certification, any laboratory which analyzes drinking water compliance samples is considered a drinking water laboratory. This includes Federal laboratories that analyze compliance samples and any laboratories that analyze compliance samples for Federal facilities. All such laboratories must (40 CFR 141.21, .23, .24, .25) be certified by the State or EPA. If requested by the State, a Region may certify Federal laboratories in its Region.

The Region certifies individual laboratories on Tribal Nation lands, when requested by the tribal chairperson. These laboratories must meet the criteria for certification as specified in the NPDWR.

The Regions use the criteria, procedures, and mechanism as specified in the NPDWR and this manual in their decisions to certify municipal or private drinking water laboratories in non-primacy states.

10. Other Considerations for Laboratory Certification

10.1 Laboratory Personnel

The laboratory should have sufficient supervisory and other personnel, with the necessary education, training, technical knowledge, and experience for their assigned functions.

10.2 Laboratory Director/Manager or Technical Director

The laboratory director/manager should be a qualified professional with the technical education and experience, and managerial capability commensurate with the size/type of the laboratory. The laboratory director/manager is ultimately responsible for ensuring that all laboratory personnel have demonstrated proficiency for their assigned functions and that all data reported by the laboratory meet the required quality assurance (QA) criteria and regulatory requirements.

10.3 Quality Assurance Manager

The QA manager should be independent from the laboratory management, if possible, and have direct access to the highest level of management. The QA manager should have a bachelor's degree in science, training in quality assurance principles commensurate with the size and sophistication of the laboratory, and at least one year of experience in quality assurance. The QA manager should have at least a working knowledge of the statistics involved in quality control of laboratory analysis and a basic understanding of the methods which the laboratory employs.

11. Laboratory Quality Assurance Plan

All laboratories analyzing drinking water compliance samples must adhere to any required QC procedures specified in the methods. This is to ensure that routinely generated analytical data are scientifically valid and defensible, and are of known and acceptable precision and accuracy. To accomplish these goals, each laboratory should (EPA Order 5360.1 A2) prepare a written description of its QA activities (a QA plan). It is the responsibility of the QA manager to keep the QA plan up to date. All laboratory personnel need to be familiar with the contents of the QA plan. This plan should be submitted to the auditors for review prior to the on-site visit or should be reviewed as part of the on-site visit.

The laboratory QA plan should be a separately prepared text. However, documentation for many of the listed QA plan items may be made by reference to appropriate sections of this manual, the laboratory's standard operating procedures, (SOPs) or other literature (e.g., promulgated methods, *Standard Methods for the Examination of Water and Wastewater*, etc.). The QA Plan should be updated at least annually (EPA Order 5360.1 A2).

At a minimum, the following items should be addressed in each QA plan:

11.1 Laboratory organization and responsibility

- include a chart or table showing the laboratory organization and lines of responsibility, including QA managers;
- list the key individuals who are responsible for ensuring the production of valid measurements and the routine assessment of measurement systems for precision and accuracy (e.g., who is responsible for internal audits and reviews of the implementation of the plan and its requirements);
- reference the job descriptions of the personnel and describe training to keep personnel updated on regulations and methodology, and document that laboratory personnel have demonstrated proficiency for the methods they perform.

11.2 Process used to identify clients' Data Quality Objectives

11.3 SOPs with dates of last revision

- The laboratory should maintain SOPs that accurately reflect all phases of current laboratory activities
- keep a list of SOPs
- ensure that current copies of SOPs are in the laboratory and in the QA Managers files;
- ensure that SOPs are reviewed annually and revised as changes are made;
- ensure that SOPs have signature pages and revisions dated.

11.4 Field sampling procedures

- describe the process used to identify sample collectors, sampling procedures and locations, required preservation, proper containers, correct sample container cleaning procedures, sample holding times from collection to analysis, and sample shipping and storage conditions;
- ensure that appropriate forms are legibly filled out in indelible ink or hard copies of electronic data are available. See Chapters IV, V, and VI for specific items to be included;
- describe how samples are checked when they arrive for proper containers and temperature and how samples are checked for proper preservation (e.g., pH, chlorine residual) before analysis;
- ensure that sampling protocol is written and available to samplers.

11.5 Laboratory sample receipt and handling procedures

- bound laboratory note books, if used, should be filled out in ink; entries dated and signed (A secure, password protected, electronic data base is acceptable);
- store unprocessed and processed samples at the proper temperature, isolated from laboratory contaminants, standards and highly contaminated samples and, sometimes, each other; holding times may not be exceeded;
- maintain integrity of all samples, (e.g., by tracking samples from receipt by laboratory through analysis to disposal);
- require Chain-of-Custody procedures for samples likely to be the basis for an enforcement action (see Appendix A);
- specify criteria for rejection of samples which do not meet shipping, holding time and/or preservation requirements and procedures for notification of sample originators.

11.6 Instrument calibration procedures (may reference SOP)

- specify type of calibration used for each method and frequency of use;
- describe calibration standards' source, age, storage, labeling;
- perform data comparability checks;
- use control charts and for radiochemistry, report counting errors with their confidence levels.

11. 7 Analytical procedures (may reference SOP)

- cite complete method manual;
- describe quality control procedures required by the methods that need to be followed.

11.8 Data reduction, validation, reporting and verification (may reference SOP)

- describe data reduction process: method of conversion of raw data to mg/L, picocuries/L, coliforms/100 mL, etc.;
- describe data validation process;
- describe reporting procedures, include procedures and format;
- describe data verification process;
- for radiochemistry, describe reporting of counting uncertainties and confidence levels;
- describe procedure for data corrections.

11.9 Type of quality control (QC) checks and the frequency of their use (see Chapters IV, V and VI).(may reference SOP)

Parameters for chemistry and radiochemistry should include or reference:

- instrument performance check standards;
- frequency and acceptability of method detection limit (MDL) calculations;
- frequency and acceptability of demonstration of low level capability;
- calibration, internal and surrogate standards;
- laboratory reagent blank, field reagent blank and trip blank;
- field and laboratory matrix replicates;
- quality control and proficiency testing samples;
- laboratory fortified blank and laboratory fortified sample matrix replicates;
- initial demonstration of method capability

- use of control charts;
- qualitative identification/confirmation of contaminants.

Parameters for microbiology should include or reference:
- positive and negative culture controls;
- confirmation/verification of presumptive total coliform positive samples;
- sterility controls;
- proficiency testing and quality control samples.

11.10 List schedules of internal and external system and data quality audits and inter laboratory comparisons (may reference SOP)

11.11 Preventive maintenance procedures and schedules
- describe location of instrument manuals and schedules and documentation of routine equipment maintenance;
- describe availability of instrument spare parts in the laboratory;
- list any maintenance contracts in place.

11.12 Corrective action contingencies
- describe response to obtaining unacceptable results from analysis of PT samples and from internal QC checks;
- name persons responsible for the various corrective actions;
- describe how corrective actions taken are documented;

11.13 Record keeping procedures
- describe procedures and documentation of those procedures;
- list length of storage, media type (electronic or hard copy);
- describe security policy of electronic databases;
- all electronic data should have software support so it may be regenerated.

If a particular item is not relevant, the QA plan should state this and provide a brief explanation. A laboratory QA plan should be responsive to the above items while remaining brief and easy to follow. Minimizing paperwork, while improving dependability and quality of data, are the intended goals.

12. Chain-of-Custody Procedures

Certified laboratories, when requested to process a sample for possible legal action against a supplier, should use an adequate chain-of-custody procedure. An example of such a procedure is found in Appendix A. The State or Region should seek input from its attorney general's office to ensure that the laboratory's procedures are adequate. The procedure used should be documented.

13. Requirements for Maintaining Certification Status

13.1 Proficiency Testing (PT) Samples

At least annually drinking water laboratories certified for chemical contaminants must satisfactorily analyze a PT sample to maintain certification (40CFR 141.23(k)(3)(i),141.24(h)(17)(i)(A) and 141.89(a)(1)(i)). PT samples should be analyzed in the same manner as routine samples. Laboratories must acquire the PT sample from a supplier acceptable to the appropriate certification authority.

If the certified laboratory does not analyze the PT sample within the acceptance limits specified in the regulations, or within policy described by their certifying authority, the certifying authority should follow the procedure discussed in the section entitled, "Criteria and Procedures for Downgrading/Revoking Certification Status."

If a laboratory wishes to be certified for a contaminant by more than one method, it must (40CFR 141.23(k)(3)(ii),141.24(h)(17)(i)(A) and 141.89(a)(1)(i)) analyze the PT samples by each method for which it wishes to be certified. The methods listed on the laboratory's certification certificate must be the methods by which the PT samples were analyzed.

The laboratory should be able to provide documentation to the certification authority that the person(s) analyzing any PT sample is a laboratory employee who routinely analyzes drinking water compliance samples.

13.2 Methodology

Laboratories must use the methods specified in the drinking water regulations at 40 CFR part 141 These methods are listed in Chapters IV, V, VI, and Appendix G.

13.3 On-Site Evaluation

The CA should be satisfied that a laboratory is maintaining the required standard of quality for certification. Normally, this should be based on a recommendation from a triennial on-site evaluation. However, if the laboratory undergoes a major change or repeatedly fails a PT sample, the CA should consider conducting an evaluation before the usual three year period has expired.

13.4 Notification of Certifying Authority (CA) of Major Changes

Certified laboratories should notify the appropriate CA (Regional Administrator or designee or the Chief, TSC-OGWDW) **in writing**, within 30 days of major changes in personnel, equipment, or laboratory location. A major change in personnel is defined as the loss or replacement of the laboratory supervisor or a situation in which a trained and experienced analyst is no longer available to analyze a particular parameter for which certification has been granted. The CA should discuss the situation with the laboratory supervisor and establish a schedule for the laboratory to address major changes. If the CA determines that the laboratory can no longer produce valid data, the CA should follow the procedure for revocation of certification.

14. Criteria and Procedures for Downgrading/Revoking Certification Status

14.1 Criteria for Downgrading Certification Status

A laboratory should be downgraded to "provisionally certified" status for a contaminant or group of contaminants for any of the following reasons:

- Failure to analyze a PT sample at least annually within the acceptance limits specified in the regulations, or, if there are no requirements specified in the regulations, within policy described by their certifying authority;
- Failure of a certified laboratory to notify the CA within 30 days of major changes (e.g., in personnel, equipment, or laboratory location);
- Failure to satisfy the CA that the laboratory is maintaining the required standard of quality, based upon a EPA on-site evaluation;
- Failure to report compliance data to the public water system or the State drinking water program in a timely manner, thereby preventing compliance with Federal or State regulations and endangering public health. Data which may cause the system to exceed an MCL should be reported as soon as possible.

14.2 Procedures for Downgrading to "Provisionally Certified" Status

If a laboratory is subject to downgrading on the basis of the above indicated criteria, the CA should notify the laboratory director or owner (by registered or certified mail) of its intent to downgrade within 14 days from becoming aware of the situation warranting downgrading. The laboratory director should review the problems cited, and within 30 days of receipt of the letter, send a letter to the CA specifying what immediate corrective actions are being taken and any proposed actions that need the concurrence of the CA. The CA should consider the adequacy of the response and notify the laboratory in writing (by registered or certified mail) of its certification status within 14 days of receipt of its response. The CA should follow up to ensure that corrective actions have been taken.

If a laboratory fails to analyze a PT or other unknown sample within the acceptance limits, the CA should not downgrade certification if the laboratory identifies and corrects the problem to the CA's satisfaction within 30 days of being notified of the failure. If, after a review of the submitted information, the CA determines that the laboratory need not be downgraded, then within 30 days of this decision, the CA should notify the laboratory that it is required to analyze

another PT. If the laboratory analyzes this second unknown sample within the acceptance limits established by the EPA or State, the laboratory should not be downgraded. If the laboratory fails to analyze this second unknown sample within the established limits, the CA should downgrade the laboratory to "provisionally certified" status and notify the laboratory within 14 days (by registered or certified mail). Laboratories should be downgraded only for the analyte failed, except where EPA/State certifies a group of related analytes based on a limited number of analytes in the group. (See Chapter 4, Section 7.2.1 for additional information.)

During any phase of this procedure, a laboratory may request that the EPA or State provide technical assistance to help identify and resolve any problem.

After the CA notifies a laboratory, in writing, that it has been downgraded to "provisionally certified" status for procedural, administrative, equipment or personnel deficiency, the laboratory should correct its problem within three months. If the laboratory was downgraded to "provisionally certified" status because of a failure to analyze a PT sample (or other unknown test sample) within the acceptance limits specified in the regulations, or within policy required by their certifying authority, the laboratory should correct its problems and satisfactorily analyze another PT sample (or other unknown sample) within one month of receipt of the second PT sample. A provisionally certified laboratory may continue to analyze samples for compliance purposes, but should notify its clients of its downgraded status and provide that information, in writing, on any report.

14.3 Criteria for Revoking Certification Status

A laboratory should be downgraded from certified, provisionally certified or interim certified status to "not certified" for a particular contaminant analysis for the following reasons:

- Reporting PT data from another laboratory as its own;
- Falsification of data or other deceptive practices;
- Failure to use the analytical methodology specified in the regulations;
- For provisionally certified laboratories, failure to successfully analyze a PT sample or any other unknown test sample for a particular contaminant within the acceptance limits specified;
- For provisionally certified laboratories, failure to satisfy the CA that the laboratory has corrected deviations identified during an on-site evaluations;
- For provisionally certified laboratories, persistent failure to report compliance data to the public water system or the State drinking water program in a timely manner thereby preventing compliance with Federal and/or State regulations and endangering public health. Data which may cause the system to exceed an MCL should be reported as soon as possible.
- Refusal to participate in an on-site evaluation conducted by the CA

14.4 Procedures for Revocation

The CA should notify the laboratory, in writing (by registered or certified mail) of the intent to revoke certification. If the laboratory wishes to challenge this decision, a notice of appeal should be submitted in writing to the CA within 30 days of receipt of the notice of intent to revoke certification. If no notice of appeal is filed, certification should be revoked.

The notice of appeal should be supported with an explanation of the reasons for the challenge and should be signed by a responsible official from the laboratory such as the president/owner for a commercial laboratory, or the laboratory supervisor in the case of a municipal laboratory or the laboratory director for a State or Regional laboratory.

Within 30 days of receipt of the appeal, the CA should make a decision and notify the laboratory in writing (by registered or certified mail). Denial of the appeal should result in immediate revocation of the laboratory's certification. Once certification is revoked, a laboratory may not analyze drinking water samples for compliance until its certification has been reinstated.

If the appeal is determined to be valid, the CA should take appropriate measures to reevaluate the facility and notify the laboratory, in writing (by registered or certified mail), of its decision within 30 days of the reevaluation.

14.5 Upgrading or Reinstatement of Certification

Through a written request, a laboratory may seek upgrading or reinstatement of certification, when and if the laboratory can demonstrate to the CA's satisfaction that the deficiencies which produced provisionally certified status or revocation have been corrected. This may include an on-site evaluation, successful analysis of unknown samples or any other measure the CA deems appropriate.

15. Record Keeping

The certification program manager should ensure that records for on-site laboratory assessments and certification program reviews be maintained in an easily accessible central location for a period of 6 years to include the last two on-site audits, or longer if required by specific State regulations. This includes records/correspondence used to determine compliance with the requirements in this manual. Records may include checklists, corrective action reports, final reports, certificates, PT study results and related documents

16. Reciprocity

Reciprocity (mutually acceptable certification among Regions and/or primacy States) is strongly endorsed by EPA as a highly desirable element in the certification program for drinking water laboratories.

States are encouraged to adopt provisions in their laws and regulations to permit reciprocity. Even though ultimate responsibility for reciprocal certification resides with the Regions and primacy States, the States may ask for the assistance of EPA in cases involving clarification of what should be considered in a reciprocal agreement. Such requests should be submitted to the Region or OGWDW through the Region.

17. Training

Training is an integral part of the laboratory certification process for personnel conducting on-site evaluations of laboratories on behalf of either the Regional Office or a primacy State.

EPA policy requires that all Regional Certification Officers initially pass the appropriate EPA laboratory certification training courses for the discipline for which they certify (chemistry or microbiology). All laboratory auditors should be experienced professionals, and have at least a bachelor's degree or equivalent education/experience in the discipline for which they certify and recent laboratory experience in the field for which they audit laboratories. Third party auditors (see Appendix D) also need to pass the EPA certification training course. Outside experts, retained for their knowledge in a limited area (e.g., asbestos) are not required to pass the laboratory certification course if they are used as part of an on-site audit team which includes a certification officer. Periodic training for both laboratory auditors and analysts should be provided by the Regions. Certification officers should attend refresher training programs at least every five years to keep their knowledge of the methods and the drinking water program current. It is highly recommended that certification officers have recent bench experience in the methods for which they certify. OGWDW will notify certification officers of major updates/changes to EPA's certification program. It is recommended that the States use these same criteria in their certification programs.

18. Alternate Test Procedures (ATPs)

EPA promulgates analytical methods for all regulated drinking water contaminants. A regulation for a particular contaminant will include one or more methods that must be used to determine that contaminant. Subsequently, the Agency may approve additional methods or modifications of EPA approved methods in another rule. EPA may also authorize the use of alternate analytical methods as provided in 40 CFR 141.27, "With the written permission of the State, concurred by the Administrator of the EPA, an alternate analytical technique may be employed. An alternate technique may be accepted only if it is substantially equivalent to the prescribed test in both precision and accuracy as it relates to the determination of compliance with any MCL."

Anyone can request that EPA approve a new method or modification of a method already approved by EPA, by submitting EPA-specified data and other information to the Director, Analytical Methods Staff,(MS 4303T) Office of Science and Technology, Office of Water, EPA, 1200 Pennsylvania Ave., NW, Washington DC 20460. EPA will evaluate the material to determine whether the method or method modification meets EPA criteria.

In the case of "acceptable versions" of methods, (minor modifications to approved methods), a letter of approval will be issued by OW. A list of these approved minor modifications can be found on the OW website at http://www.epa.gov/OGWDW/methods.

Chapter IV
Critical Elements for Chemistry

1. Personnel

1.1 Laboratory Supervisor

The laboratory supervisor should have at least a bachelor's degree with a major in chemistry or equivalent, and at least one year of experience in the analysis of drinking water. The laboratory supervisor should have at least a working knowledge of quality assurance principles. The laboratory supervisor has the responsibility to ensure that all laboratory personnel have demonstrated their ability to satisfactorily perform the analyses to which they are assigned and that all data reported by the laboratory meet the required quality assurance and regulatory criteria.

1.2 Laboratory Analyst

The laboratory analyst should have at least a bachelor's degree with a major in chemistry or equivalent, and at least one year of experience in the analysis of drinking water. If the analyst is responsible for the operation of analytical instrumentation, he or she should have completed specialized training offered by the manufacturer or another qualified training facility or served a period of apprenticeship under an experienced analyst. The duration of this apprenticeship should be proportional to the sophistication of the instrument. Data produced by analysts and instrument operators while in the process of obtaining the required training or experience are acceptable only when reviewed and validated by a fully qualified analyst or the laboratory supervisor.

Before beginning the analysis of compliance samples, the analyst must adhere to any required QC procedures specified in the methods for blanks, precision, accuracy, sensitivity, specificity and satisfactory analysis on unknown samples. This should be documented according to the laboratory's QA Plan.

1.3 Technician

The laboratory technician should have at least a high school diploma or equivalent, complete a method training program under an experienced analyst and have six months bench experience in the analysis of drinking water samples.

Before beginning the analysis of compliance samples, the technician must adhere to any required QC procedures specified in the methods for blanks, precision, accuracy, sensitivity, specificity and satisfactory analysis on unknown samples. This should be documented according to the laboratory's QA Plan.

1.4 Sampling Personnel

Personnel who collect samples should be trained in the proper collection technique for all types of samples which they collect. Their technique should be reviewed by experienced sampling or laboratory personnel.

1.5 Waiver of Academic Training Requirement

The certification officer may waive the need for specified academic training, on a case-by-case basis, for highly experienced analysts.

1.6 Training Records

Training records should be maintained for all personnel. These should include all job-related formal education and training taken by the analyst which pertains to any aspect of his/her responsibilities, including but not limited to analytical methodology, laboratory safety, sampling, quality assurance, data analysis, etc.

2. Laboratory Facilities

The analysis of compliance samples is to be conducted in a laboratory where the security and integrity of the samples and the data can be maintained. The laboratory facilities should be clean, have adequate temperature and humidity control, have adequate lighting at the bench top and should meet applicable OSHA standards. The laboratory must adhere to any required QC procedures specified in the methods by having provisions for the proper storage and disposal of chemical wastes; secondary containment for hazardous waste storage is recommended. The appropriate type of exhaust hood is required where applicable.

There should be sufficient bench space for processing samples. Workbench space should be convenient to sink, water, gas, vacuum and electrical sources free from surges. Instruments should be properly grounded. For safety reasons, inorganic and organic facilities should be in separate rooms; organic analysis and sample extraction should also be separated to prevent cross contamination. The analytical and sample storage areas should be isolated from all potential sources of contamination. There should be sufficient storage space for the safe storage of chemicals, glassware and portable equipment, sufficient floor and bench space for stationary equipment and areas for cleaning materials.

3. Laboratory Equipment and Instrumentation

The laboratory is to have the instruments and equipment needed to perform the approved methods for which certification has been requested. The checklist on pages 44 to 50 of this chapter provides more information on the necessary equipment. All instruments are to be properly maintained and calibrated.

4. General Laboratory Practices

4.1 General

4.1.1 Chemicals/reagents: Chemicals and reagents used must meet any requirements specified in the methods. If not specified, then "Analytical reagent grade" (AR) or American Chemical Society (ACS) grade chemicals or better should be used for analyses in certified laboratories. Consult the currently promulgated editions of *Standard Methods for the Examination of Water and Wastewater*, part 1070 for more detailed information on reagent grades.

4.2 Inorganic Contaminants

4.2.1 Reagent water: The laboratory must have a source of reagent water having a resistance value of at least 0.5 megohms (conductivity less than 2.0 micromhos/cm) at 25°C when required by the method. High quality water meeting such specifications may be purchased from commercial suppliers. Quality of reagent water is best maintained by sealing it from the atmosphere. Quality checks to meet specifications above should be made and documented at planned intervals based on use. Individual analytical methods may specify additional requirements for the reagent water to be used. Inorganic methods require distilled or deionized water free of the analyte(s) of interest and trace metals methods require ASTM Type 1 water.

4.2.2 Glassware preparation: Glassware cleaning requirements specified in the methods must be followed. If no specifications are listed, then glassware should be washed in a warm detergent solution and thoroughly rinsed first with tap water and then with reagent water. This cleaning procedure is sufficient for general analytical needs. It is advantageous to maintain separate sets of suitably prepared glassware for the nitrate and mercury analyses due to the potential for contamination from the laboratory environment. Table IV-1 summarizes the cleaning procedures specified in the EPA methods.

4.3 Organic Contaminants

4.3.1 Reagent water: Reagent water for organic analysis must adhere to any required QC specified in the methods. Most methods specify the reagent water not contain analytes of interest above their respective method detection levels (MDLs). It may be necessary to treat water with activated carbon to eliminate all interferences. Reagent water requirements of individual methods must be followed.

4.3.2 Glassware preparation: Glassware cleaning requirements specified in the methods must be followed. Table IV-1 summarizes the cleaning procedures specified in the EPA methods.

4.4 Laboratory Safety

While safety criteria are not an aspect of laboratory certification, laboratory personnel should apply general and customary safety practices as a part of good laboratory practices. Each laboratory is encouraged to have a safety plan as part of their standard operating procedure which includes personnel safety, training and protection. Where safety practices are required in an approved method (i.e., 515.1), they must be followed. See *Standard Methods for the Examination of Water and Wastewater*, part 1090 for a discussion of laboratory safety.

4.5 Quality Assurance

Laboratories should maintain current Quality Assurance Plans as described in Chapter 3. All laboratory activities

including, but not limited to, sampling, test methods, instrument operation, data generation, data validation and corrective action procedures should be described in the Plan. Plans need to be read by all personnel.

5. Analytical Methods

5.1 General

A list of promulgated methods for inorganic and organic contaminants can be found in Tables IV-2 and IV-3, respectively. Methods manuals should be available to applicable personnel. Other methods cannot be used for compliance samples unless approval has been granted by the Agency by obtaining an Alternate Test Procedure approval. *Allowed* modification to the methods must be documented. Contact the appropriate certifying authority for the alternate test procedure process (see Chapter 3, p 10). Table IV-4 lists the methods which must be used for the analysis of disinfectant residuals. Recommended methods for Secondary contaminants are listed in Table IV-5.

5.2 Analyses Approved by the State

Measurements for turbidity, pH, temperature, disinfectant residual, calcium, orthophosphate, silica, alkalinity, and conductivity need not be made in certified laboratories, but may be performed by any persons acceptable to the State. However, approved methodology must) be used (Tables IV-2 to IV-5). The State should institute a quality assurance program to assure validity of data from these measurements.

5.2.1 Turbidity standards: Sealed liquid secondary turbidity standards purchased from the instrument manufacturer or other sources should be calibrated against properly prepared and diluted formazin or styrene divinylbenzene polymer primary standards and revised values assigned at least every four months in order to monitor for any deterioration. This calibration should be documented. These standards should be replaced when they do not fall within 15% of the initial assigned concentration of the standard. Solid turbidity standards composed of plastic, glass, or other materials are not reliable and should not be used.

5.2.2 Residual chlorine standards: If visual comparison devices such as color wheels or sealed ampules are used for determining free chlorine residual, the standards incorporated into such devices should be calibrated at least every six months. These calibrations need to be documented. Directions for preparing temporary and permanent type visual standards can be found in Method 4500-Cl-G, of the currently promulgated editions of *Standard Methods for the Examination of Water and Wastewater.* By comparing standards and plotting such a comparison on graph paper, a correction factor can be derived and applied to future results obtained on the now calibrated apparatus.

6. Sample Collection, Handling, and Preservation

The manner in which samples are collected and handled is critical to obtaining valid data. It is important that a written sampling protocol with specific sampling instructions be available to and used by sample collectors and available for inspection by the certification officer. (Appendix A, Chain-of-Custody).

6.1 Rejection of Samples

The laboratory's rejection criteria should be documented in writing in the laboratory's QA Plan or in an SOP. The laboratory should reject any sample taken for compliance purposes which does not meet the criteria in 6.2 through 6.6. The laboratory must (141.23(a)(4)(i))notify the authority requesting the analyses and ask for a resample. If resampling is not possible and the sample is analyzed, the sample data should be clearly identified in the data package as being unusable for its intended purpose. In addition, the inadmissibility of these sample data need to be clearly communicated to all end data users.

6.2 Sample Containers and Preservation

The type of sample container and the required preservative for each inorganic and organic chemical contaminant are listed in Table IV-6. The laboratory must measure and record the temperature of the sample when it arrives when temperature preservation is required by the method. The use of "blue ice" is discouraged because it generally does not maintain the temperature of the sample at 4°C ±2°C or less. If blue ice is used, it should be frozen at the time of sampling, the sample should be chilled before packing, and special notice taken at sample receipt to be certain the required temperature (4°C) has been maintained.

6.3 Maximum Holding Times
Samples must be analyzed within the maximum holding times required by the method. These are listed in Table IV-6.

6.4 Sample Collection and Transport
There must be strict adherence to correct sampling procedures, sample handling, complete identification of the sample, and prompt transfer of the sample to the laboratory when required by the method. When the laboratory is not responsible for sample collection and transport, it must verify that the paperwork, preservatives, containers and holding times are correct as required by the methods or reject the sample. The rejection criteria should (EPA Order 5360.1) be documented in writing.

6.5 Sample Collector
The sample collector should be trained in sampling procedures and have complete written sampling instructions (SOPs) for each type of sample to be collected. The samplers are to be able to demonstrate proper sampling technique.

6.6 Sample Report Form
The sample collection report form should contain, at a minimum, the ID, location, date and time of collection, collector's name, preservative added and shipping requirements, container and volume, sample type, analysis, and any special remarks concerning the sample. Indelible ink should be used.

6.7 Sample Compositing
If samples are composited, the compositing must (40 CFR 141.23,24) be done in the laboratory. Samples may only be composited if the laboratory detection limit is adequate for the number of samples being composited (up to a maximum of five). For example, for inorganic samples, composite samples from a maximum of five samples are allowed if the detection limit of the method used for analysis is less than one-fifth the MCL. If the concentration of any inorganic chemical in the composite is greater than or equal to one-fifth of the MCL, then a followup sample must be taken within 14 days at each sampling point included in the composite. These samples must be analyzed for the contaminants which exceeded one-fifth the MCL in the composite sample. [CFR 144.23(a)(4)] Compositing of VOCs is not recommended.

7. Quality Control
7.1 General Requirements

7.1.1 Availability of QA Documents: The laboratory's QA plan and appropriate Standard Operating Procedures (SOPs) should be readily available to the analysts and for inspection by auditors. (see Chapter III's discussion of Quality Assurance).

7.1.2 Availability of QC Information: All quality control information should be readily available for inspection by auditors.

7.1.3 Balances and Weights: Balance range should be appropriate for the application for which it is to be used. Drinking water chemistry laboratories should use balances that weigh to at least 0.0001 g. The balances should be calibrated at least annually with ASTM Type I, Class 1 or 2 weights. (ASTM, 1916 Race St., Philadelphia, PA 19103) This may be done by laboratory personnel or under contract by a manufacturer's representative. We strongly recommend that laboratories have a contract to calibrate balances due to the expense of the calibration weights, and to serve as an outside QC check of the weights and balances. Weights meeting ASTM Type I, Class 1 or 2 specifications should be recertified at least every five years or if there is reason to believe damage (corrosion, nicks) has occurred.

Each day the mechanical or digital balance is used, a verification should be performed. The verification consists of a check of a reference mass at approximately the same nominal mass to be determined. Verifications should be done each weighing session unless it can be shown that fluctuations in the environment do not affect the calibration. Weights meeting ASTM Type 1 specifications may be used. These should be calibrated annually against the reference weights at time of balance calibration. The checks and their frequency should be as prescribed in the laboratory's QA Plan. A record of all checks should be kept and be available for inspection.

7.1.4 Color Standards: Wavelength settings on spectrophotometers should be verified at least annually with color standards. The specific checks and their frequency should be as prescribed in the laboratory's QA documents. A record of these checks should be kept as prescribed in the laboratory's QA documents and be available for inspection.

7.1.5 Temperature Measuring Devices Liquid bearing thermometers such as mercury or alcohol thermometers need to be traceable to NIST calibration and verified at least annually and whenever the thermometer has been exposed to temperature extremes. The correction factor should be indicated on the thermometer and the date the thermometer was calibrated and the calibration factor should be kept as prescribed in the laboratory's QA documents and be available for inspection. The NIST thermometer should be recalibrated at least every five years or whenever the thermometer has been exposed to temperature extremes.

Digital thermometers, thermocouples and other similar electronic temperature measuring devices should be calibrated at least quarterly. The date the thermometer was calibrated and the calibration factor should be kept as prescribed in the laboratory's QA documents and be available for inspection.

When an infrared detection device is used to measure the temperature of samples, the device should be verified at least every six months using a NIST certified thermometer over the full temperature range that the IR thermometer will be used. This would include ambient (20-30°C), iced (4°C) and frozen (0 to -5°C). Each day of use a single check of the IR should be made by checking the temperature of a bottle of water at the temperature of interest that contains a calibrated thermometer. Agreement between the two should be within 0.5°C, or the device should be recalibrated.

7.1.6 Traceability of Calibration: Calibrations of all measurement devices need to be traceable to national standards whenever applicable.

*7.2 **Specific Requirements:*** The following are required for each analyte for which a laboratory is certified:

7.2.1 Proficiency Testing (PT) Samples: In order to receive and maintain full certification for an analyte, the laboratory must (40CFR 141.23(k)(3)(i),141.24(h)(17)(i)(A) and 141.89(a)(1)(i)) analyze PT samples (if available) acceptable to the Certifying Authority at least once every 12 months for each analyte and by each method used to analyze compliance samples. Results from analysis of the PT sample must be within the acceptable limits established by U.S. EPA. These acceptance limits are listed in Table IV-10, "MCL and Profeciency Testing Sample Acceptance Criteria in the CFR, Primary and Secondary Drinking Water Regulations [§141.23(k)(3)(ii) and 141.24(f)(17) and (19)]." The laboratory should document the corrective actions taken when a PT sample is analyzed unsuccessfully. A copy of this documentation should be available for review by the certification officer. A make up PT sample must be successfully analyzed. If problems arise, the appropriate action to be taken is specified in Chapter III, Implementation of Certification Program.

Excluding vinyl chloride, the laboratory may be certified for all VOCs if they successfully analyze at least 80% of the regulated VOCs (141.24(17)(f)(i)(B). The intention of this regulation is to allow some flexibility for random misses because the VOC methods include 20 regulated analytes. A laboratory should not be certified for an analyte which it fails repeatedly. This "80% rule" for VOCs has recently been made more difficult to interpret since some PT providers are including THMs in the same vial as the VOCs. The 80% Rule does not apply to the THMs.

The Stage 1 Disinfection By Products (DBP) Rule, which became effective in January 2002, regulates the sum of five haloacetic acids (HAA5): monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid and dibromoacetic acid. Laboratories are certified for HAA5, but successful analyses of the HAA PT samples are based on the results for the individual compounds. The 80% Rule applies to the HAA5s, so if four of the 5 HAA5s are successfully analyzed, the laboratory may be certified for HAA5. As before, a laboratory should not be certified if the same analyte is failed repeatedly.

The DBP Rule also changed the way the trihalomethanes (THMs): chloroform, dichlorobromomethane, chlorodibromomethane and bromoform PTs are evaluated. Laboratories are still certified for total THMs but under the DBP Rule, each THM concentration must (141.131(b)(2) be reported, evaluated and passed individually to pass the PT sample. The DBP Rule also states that if a laboratory fails one THM, it cannot be certified for TTHMs, but must (141.131(b)(2)analyze another PT sample and pass all four of the THMs in a PT sample to be certified to analyze compliance monitoring samples for total trihalomethanes.

The following table summarizes the 80% Rule.

Analyte(s)	PT Success Requirement
Vinyl Chloride	100%
20 VOCs	80% [1]
4 THMs	100%
5 HAA5s	80% [1]

[1] A lab should not maintain certification for analyte(s) which it repeatedly fails.

7.2.2 Quality Control Samples: At least once each quarter, the laboratory should analyze a quality control sample for the analytes they are determining in that quarter. The sample should be prepared from a source other than that from which their working standards are prepared. The sample should be in the same concentration range as the drinking water calibration curve. If errors exceed limits required in the methods, corrective action must be taken and documented, and a follow-up quality control sample analyzed as soon as possible to demonstrate the problem has been corrected.

7.2.3 Calibration Curve: Calibration requirements in the methods must be followed. If there are no calibration requirements in the method, the following are guidelines to be used. At the beginning of each day that samples are to be analyzed, a calibration curve covering the sample concentration range and all target analytes should be generated according to the approved SOP. Depending on concentration ranges, the curve should be composed of three or more points. Field measurements (e.g. pH and chlorine residual) need to be made on instruments which have been properly calibrated as specified in the method or instrument manual and checked each day of use. The less precise the measurement, the greater the number of concentrations which should be included in the calibration curve.

7.2.4 Calibration Check: The calibration for some methods is so time-consuming that 7.2.3 is impractical on a daily basis. Where the determinative time is extensive such as Methods 508/508.1, 515.1, 524.2, 525.2, etc. and the instrument is very stable, the calibration curve should be initially developed as specified in 7.2.3. Thereafter, each day analyses are performed, this curve should be verified by analysis of at least one standard for each of the target analytes at the expected concentration range. This verification should be done at both the beginning and end of the analyses. All checks must be within the control limits required in the method or the system is to be recalibrated as specified in 7.2.3. The concentration of the check standard should vary from day to day across the range of analyte concentrations being measured.

For some methods an initial conditioning injection is to be made to deactivate active sites that may have developed overnight. Depending on the method, the blank may be appropriate for this.

Specific calibration requirements in the methods must be followed if different than the above.

It is recommended that a calibration standard of one component of a multicomponent analyte (PCBs, toxaphene or chlordane) also be analyzed each day or work shift. By rotating the analyte chosen, continuing calibration data can be obtained on all the multicomponent analytes over a period of one to two weeks. If a positive for a multcomponent analyte is found in a sample, a calibration check for that analyte should be performed as soon as possible.

7.2.5 Blanks: Requirements in the methods must be followed. A laboratory reagent blank should be carried through the full analytical procedure with every sample batch. In general, results from laboratory reagent blanks should not exceed the laboratory's Minimum Reporting Limit, the lowest concentration of standard used for quantitation. (MRL).

7.2.6 Laboratory Fortified Blanks: Requirements in the methods must be followed. LFBs should be analyzed at the level specified in the method. Some methods require that a laboratory fortified blank at ten times the MDL or a mid level concentration be analyzed with each batch of samples. Precision and accuracy data should be documented for this determination. In addition, the analyst should routinely verify the minimum reporting limit for each analyte by analyzing a laboratory fortified blank at the minimum reporting level.

7.2.7 Laboratory Fortified Sample Matrix: Laboratory fortified sample matrix requirements in the methods must be met. If there are no laboratory fortified sample matrix requirements in the method, the following are guidelines to be used. The laboratory should add a known quantity of analytes to a percentage (to be described in the approved SOP) of the routine samples to determine sample matrix interference. The fortified concentration should not be less than the concentration of the sample selected for fortification unless specified by the method. If the sample concentration is unknown or less than detectable, the analyst should choose an appropriate concentration (e.g., a percentage of the MCL or mid point in the calibration range). **Over time, samples from all routine sample sources should be fortified. The procedure should be described in the SOP.** If any of these checks are not within the criteria specified in the method or control limits specified in 7.2.7, and the laboratory performance is in control, the result for that sample should be flagged to inform the data user that the results are suspect due to matrix effects.

7.2.8 Control Charts: Control charts for accuracy and precision, generated from laboratory fortified blanks (LFBs) should be maintained and used by the laboratory. Until sufficient data are available from the laboratory, usually a minimum of 20 to 30 test results on a specific analysis, the laboratory should use the control limits specified in the methods. If there are no control limits specified in the method, the limits may be statistically calibrated using the procedure below.

When sufficient data become available, the laboratory should develop LFB control charts from the mean percent recovery ($\bar{x}$) and the standard deviation (S) of the percent recovery for the QC checks specified above (see *Standard Methods for the Examination of Water and Wastewater*, part 1020B, or similar QC reference texts for further information). These data are used to establish upper and lower control limits as follows:

$$\text{upper control limit} = \bar{x} + 3S \quad (\text{upper warning limit} + 2S)$$
$$\text{lower control limit} = \bar{x} - 3S \quad (\text{lower warning limit} - 2S)$$

After each five to ten new recovery measurements, new control limits should be calculated using the most recent 20-30 data points. These calculated control limits should not exceed those established in the method. If any of these control limits are tighter than the method specifications, the laboratory should use the tighter criteria.

7.2.9 Initial Demonstration of Capability: Requirements in the methods must be followed. Before beginning the analysis of compliance samples, an initial demonstration of capability (IDC) must be performed for each method as required in the method. The IDC includes a demonstration of the ability to achieve a low background, the precision and accuracy required by the method, and determination of the method detection limit (MDL)(see below). An IDC should be performed for each instrument. It is also recommended that an IDC be performed by each analyst. In addition, it is recommended that the IDC also address the variability introduced if more than one sample preparation technician is used. Precision, accuracy and MDL should be similar for each technician. The analyst should recalculate IDCs when a change in the method, analyst or instrument is made which could affect the precision or accuracy or sensitivity. Minor changes should prompt a check to ascertain that the precision, accuracy and sensitivity have been maintained.

7.2.10 Quantitation of Multicomponent Organic Analytes (toxaphene, chlordane and PCBs) The quantitation of multicomponent analytes requires professional judgment on the part of the analyst. This is required due to the complex nature of the chromatography involved, sample weathering, degradation and interferences that may be present in the samples. The pattern of peaks found in the sample should be examined carefully and compared to a standard. The peaks in the sample that match the peak ratios in the standard can be used in quantitation. Peaks that have obvious interferences (such as pesticides or phthalates or peaks exhibiting poor peak shape) or appear to have been degraded or weathered should not be used for quantitation. A representative number (5-9) of peaks is suggested. Peak area should be used for quantitation and the analyst should ensure that the samples and standards have been integrated in the same manner. Quantitation can be done by using the total peak area or height (comparing the area of the 5-9 peaks used for quantitation of the sample to the area of the standard) or by calculating each peak separately (using area) and taking the average concentration of the 5-9 peaks. Because of factors such as peak shape and baseline rise, the most accurate quantitation is obtained when the concentration of the sample closely matches that of the standard (e.g., within 20% of the standard). See EPA Method 8081, Organochlorine Pesticides and PCBs as Aroclors by Gas Chromatography: Capillary Column Technique, (EPA SW 846 Test Methods for Evaluating Solid Waste Physical/Chemical Methods, Third Edition) for a more detailed discussion of quantitation of multicomponent analytes.

Note: PCBs are qualitatively identified as Aroclors and measured for compliance purposes as decachlorobiphenyl. Chlordane is regulated as technical chlordane, a mixture of at least 11 major components and 30 minor ones.

7.2.11 MDL Calculation: Requirements in the methods must be followed. Most methods require initial MDL calculations for all analytes and certification officers should require the laboratories to calculate their detection limits for all regulated contaminants. If there is no procedure to determine the detection limits in the method, it should be determined in accordance with the procedure given in 40 CFR 136, Appendix B. The CFR, at141.24(f)(17)(ii)(C) requires an MDL of 0.0005 mg/L be attained for VOCs, and 141.89(a)(1)(iii) requires an MDL of 0.001 mg/L be attained for lead if the lab will be processing source water composite samples. For inorganics and SOCs, a method detection limit of 1/5 of the MCL must be attained for compositing [CFR 141.23(a)(4)] and [CFR 141.24(f)(10)]. VOCs should not be composited. The SOC detection limits listed at CFR (141.24(h)(18) are required to reduce monitoring (CFR 141.24(f)(11)(iv). Table IV-8 lists the MCLs, MCLGs and MDLs, for VOCs which are in the drinking water regulations. Table IV-9 lists the SOC MCLs, MCLGs and Monitoring Triggers.

Sample preparation and analyses for the MDL calculation should be made over a period of at least three days to include day-to-day variation as an additional source of error. The analyst should determine MDLs initially, when any change is made which could affect the MDLs, or more frequently if required by the method. (Inorganic methods may require MDLs to be determined differently, and in all cases the methods must be followed.) In addition, the analyst must demonstrate low level capability on an ongoing basis through an MDL determination or repeated low level analyses (MRL).

The calculation of MDLs by the CFR procedure may not be adequate for toxaphene and chlordane because they require pattern or peak profile recognition for identification. Presently, no standard procedure exists, so it is recommended that the MDL be defined as the lowest concentration for which pattern recognition is possible. Pattern recognition is used for qualitative identification of PCBs as Aroclors. Quantitation of PCBs is achieved by conversion of PCBs to decachlorobiphenyl (DCB).

7.2.12 Low Level Quantitation: The laboratory's minimum reporting limits (MRL) should be reported to the client along with the data. The reporting limit must be below the MCL. Laboratories should **run a LFB at their MRL every analysis day and should** not report contaminants at levels less than the level at which they routinely analyze their lowest standard. While this is a scientifically sound practice, whether it is an acceptable practice will depend on State and Federal reporting requirements. It is important for users of data to understand the statistical and qualitative significance of the data. Laboratories may be required by the States to achieve a specific MDL or quantitation limit more stringent than that required by EPA.

8. Records and Data Reporting

8.1 Legal Defensibility: Compliance monitoring data should be made legally defensible by keeping thorough and accurate records. The QA plan and/or SOPs need to (EPA Order 5360.1) describe the policies and procedures used by the facility for record integrity, retention and storage. If samples are expected to become part of a legal action, chain of custody procedures should be used (See Appendix A).

8.2 Maintenance of Records: **Public Water Systems** are required to maintain records of chemical analyses of compliance samples for 10 years (40 CFR 141.33) and lead and copper for 12 years (40 CFR 141.91). The laboratory should maintain easily accessible records for five years or until the next certification data audit is complete, whichever is longer. Changes in ownership, mergers, or closures of laboratories do not eliminate these requirements. The client water system should be notified before disposing of records so they may request copies if needed. This includes all raw data, calculations, and quality control data. These data files may be either hard copy, microfiche or electronic. Electronic data should always be backed up by protected tape or disk or hard copy. If the laboratory changes its computer hardware or software, it should make provisions for transferring old data to the new system so that it remains retrievable within the time frames specified above. Data which is expected to become part of a legal action may need to be maintained for a longer period of time. Check with your legal counsel.

8.3 Sampling Records: Data should be recorded in ink with any changes lined through such that the original entry is visible. Data may also be kept electronically. Changes need to be initialed and dated. The following information should be readily available:

8.3.1 Date, location (including name of utility and PWSS ID #), site within the system, time of sampling, name, organization and phone number of the sampler, and analyses required;

8.3.2 Identification of the sample as to whether it is a routine distribution system sample, check sample, raw or finished water sample, repeat or confirmation sample or other special purpose sample;

8.3.3 Date of receipt of the sample;

8.3.4 Sample volume/weight, container type, preservation and holding time and condition on receipt;

8.3.5 pH and disinfectant residual at time of sampling (if required) (from plant records);

8.3.6 Transportation and delivery of the sample (person/carrier, conditions).

8.4 Analytical Records Data should be recorded in ink with any changes lined through such that original entry is visible. Changes need to be initialed and dated The following information should be readily available:

8.4.1 Laboratory and persons responsible for performing analysis;

8.4.2 Analytical techniques/methods used;

8.4.3 Date and time of analysis;

8.4.4 Results of sample and quality control analyses;

8.4.5 Calibration and standards information.

8.4.6 Analyst and technician Initial Demonstration of Capability documentation should be kept on file as well as results of proficiency testing.

8.5 Reconstruction of Data: Adequate information should be available to allow the auditor to reconstruct the final results for compliance samples and PT samples.

8.6 Computer Programs: Computer programs should be verified initially and periodically by manual calculations and the calculations should be available for inspection. Access to computer programs and electronic data need to be limited to appropriate personnel.

9. Action in Response to Noncompliant Laboratory Results

When a laboratory is responsible, either by contract or State policy, to report sample results which would indicate a system is out of compliance, the laboratory must (141.23(a)(4)(i)) promptly notify the proper authority so that the authority can request the water utility to resample from the same sampling location(s) immediately. See Chapter III.

Table IV-1 Glassware Cleaning Procedures - consult the method for complete details; do not over-heat volumetric glassware

Method	Washing	Drying
502.2/504/504.1/524.2	Detergent wash, rinse with tap and distilled water	105°C for 1 hour
505	Detergent wash, rinse with tap and reagent water	400°C for 1 hour or rinse with acetone
506	Rinse immediately with last solvent used, wash with hot water and detergent, rinse with tap and reagent water	400°C for 1 hour or rinse with acetone
507/508	Rinse immediately with last solvent used, wash with hot water and detergent, rinse with tap and reagent water	400°C for 1 hour or rinse with acetone
508.1	Detergent wash, rinse with tap and reagent water or solvent rinse	400°C for 2 hours
508A	No specifications, suggest the same as 515.1/515.2	no specification, suggest the same as 515.1/515.2
515.1/515.2	Rinse immediately with last solvent used, wash with hot water and detergent, rinse with dilute acid, tap and reagent water	400°C for 1 hour or rinse with acetone
515.3	Rinse immediately with last solvent used, wash with hot water and detergent, rinse with tap and reagent water	400°C for 1 hour or rinse with acetone
515.4	Wash with tap water and detergent, rinse with tap and reagent water. A solvent rinse may be necessary.	In place of solvent rinse, muffle at 400°C for 2 hours. Heat volumetrics at 120°C.
524.2	Not described in method	Not described in method
525.2	Detergent wash, rinse with tap and distilled water or solvent rinse	air dry or muffle(no specs) (suggest 400°C for 1 hour)
531.1/6610	Rinse immediately with last solvent used, wash with hot water and detergent, rinse with tap and reagent water	450°C for 1 hour or rinse with acetone

Method	Washing	Drying
547/548.1	Rinse immediately with last solvent used, wash with hot water and detergent, rinse with tap and reagent water	400°C for several hours or rinse with methanol
549.2	Rinse immediately with last solvent used, wash with hot water and detergent, rinse with tap and reagent water	130°C for several hours or rinse with methanol
550/550.1	Rinse immediately with last solvent used, wash with hot water and detergent, rinse with tap and reagent water	400°C for 15-30 minutes or rinse with acetone or pesticide quality hexane
551.1	Wash with water and detergent, rinse with tap and reagent water. Rinse caps in acetone	Vials: 400°C for 30 minutes Caps: 80°C for 1 hour
552.1, 552.2, 555	Rinse immediately with last solvent used, wash with hot water and detergent, rinse with tap and reagent water	400°C for 1 hour or rinse 3 times with acetone
1613	Rinse with solvent, sonicate with detergent for 30 minutes, rinse sequentially with methanol, hot tap water, methanol, acetone and methylene chloride	Air dry
Metals	Wash with detergent, rinse with tap water, soak 4 hours in 20% (V/V) nitric acid or dilute nitric(~8%)/ hydrochloric(~17%), rinse with reagent water	Air dry
Inorganics	Wash with detergent, rinse with tap and reagent water (use phosphate free detergent for o-phosphate analysis)	Air dry

Table IV-2 Approved Methods for Primary Inorganic Chemicals, Parameters in the Lead and Copper Rule, Sodium, and Turbidity [§141.23(k)(1)]

Contaminant	Methodology	EPA	ASTM[1]	SM[2]	Other
Antimony	ICP-MS	200.8[3]			
	Hydride-AA		D3697-92		
	AA-Platform	200.9[3]			
	AA-Furnace			3113B	
Arsenic	ICP*	200.7[3]		3120B	
	ICP-MS	200.8[3]			
	AA-Platform	200.9[3]			
	AA-Furnace		D2972-93C	3113B	
	Hydride-AA		D2972-93B	3114B	
Asbestos	TEM	100.1[4]			
	TEM	100.2[5]			
Barium	ICP	200.7[3]		3120B	
	ICP-MS	200.8[3]			
	AA-Direct			3111D	
	AA-Furnace			3113B	
Beryllium	ICP	200.7[3]		3120B	
	ICP-MS	200.8[3]			
	AA-Platform	200.9[3]			
	AA-Furnace		D3645-93B	3113B	
Bromate	IC	300.1[6]			
Cadmium	ICP	200.7[3]			
	ICP-MS	200.8[3]			
	AA-Platform	200.9[3]			
	AA-Furnace			3113B	
Chlorite	IC	300.0[7]			
	IC	300.1[6]			

*ICP will not be an acceptable procedure after 2006

Contaminant	Methodology	EPA	ASTM[1]	SM[2]	Other
Chromium	ICP	200.7[3]		3120B	
	ICP-MS	200.8[3]			
	AA-Platform	200.9[3]			
	AA-Furnace			3113B	
Cyanide	Man. Distillation followed by:		D2036-98A	4500-CN-C	
	Spec., Amenable		D2036-98B	4500-CN-G	
	Spec.Manual		D2036-98A	4500-CN-E	I-3300-85[8]
	Semi-auto	335.4[7]			
	Ion Sel. Elec.(ISE)			4500CN-F	
	Lachat				Kenda
Fluoride	Ion Chromatography	300.0[7]	D4327-91	4110B	
	Manual Distillation, SPADNS			4500F-B,D	
	Manual ISE		D1179-93B	4500F-C	
	Automated ISE				380-75WE[9]
	Auto. Alizarin			4500F-E	129-71W[9]
Mercury	Manual Cold Vapor	245.1[3]	D3223-91	3112B	
	Auto. Cold Vapor	245.2[10]			
	ICP-MS	200.8[3]			
Nitrate	Ion Chromatography	300.0[7]	D4327-97	4110B	B-1011[11]
	Auto Cd Reduction	353.2[7]	D3867-90A	4500-NO_3-F	
	Ion Selective Elec.			4500-NO_3-D	601[12]
	Man Cd Reduction		D3867-90B	4500-NO_3-E	
Nitrite	Ion Chromatography	300.0[7]	D4327-97	4110B	B-1011[11]
	Auto Cd Reduction	353.2[7]	D3867-90A	4500-NO_3-F	
	Man Cd Reduction		D3867-90B	4500-NO_3-E	
	Spectrophotometric			4500-NO_2-B	

Contaminant	Methodology	EPA	ASTM[1]	SM[2]	Other
Selenium	Hydride-AA		D3859-98A	3114B	
	ICP-MS	200.8[3]			
	AA-Platform	200.9[3]			
	AA-Furnace		D3859-93B	3113B	
Thallium	ICP-MS	200.8[3]			
	AA-Platform	200.9[3]			
Lead	AA-Furnace		D3559-96D	3113B	
	ICP-MS	200.8[3]			
	AA-Platform	200.9[3]			
Copper	AA-Furnace		D1688-90C	3113B	
	AA-Direct		D1688-90A	3111B	
	ICP	200.7[3]		3120B	
	ICP-MS	200.8[3]			
	AA-Platform	200.9[3]			
pH	Electrometric	150.1[10]	D1293-84	4500-H^+-B	
		150.2[10]			
Conductivity	Conductance		D1125-91A	2510B	
Calcium	EDTA titration			3500-Ca-B[2a]	
	EDTA titration		D511-93A	3500-Ca-D[2a]	
	AA-Direct		D511-93B	3111B	
	ICP	200.7[3]		3120B	
Alkalinity	Titration		D1067-92B	2320B	
	Elec. titration				I-1030-85[8]
Ortho-phosphate unfiltered, no digestion or hydrolysis	Color, automated ascorbic acid	365.1[7]		4500-P-F	
	Color, ascorbic acid		D515-88A	4500-P-E	
	Color, phosphomolybdate				I-1601-85[8]
	AutoSegmented Flow				I-2601-90[8]
	Auto discrete				I-2598-85[8]
	Ion Chromatography	300.0[7]	D4327-97	4110	

Contaminant	Methodology	EPA	ASTM[1]	SM[2]	Other
Silica	Color, molybdate blue;				I-1700-85[8]
	auto seg. flow				I-2700-85[8]
	Color		D859-88		
	Molybdosilicate			4500-Si-D[2a]	
	Heteropoly blue			4500-Si-E[2a]	
	Auto. molybdate reactive silica			4500-Si F[2a]	
	ICP	200.7[3]		3120B	
Temperature	Thermometric			2550B	
Sodium	ICP	200.7[3]			
	AA-Direct			3111B	
Turbidity	Nephelometric	180.1[7]		2130B	GLI Method 2[13]
	Hach				10133

Footnotes

1 *Annual Book of ASTM Standards*, Vols. 11.01 and 11.02, American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

2 *Standard Methods for the Examination of Water and Wastewater*, 18th, 19th or 20th Edition, American Public Health Association, 1015 Fifteenth Street NW, Washington, D.C. 20005. Except 3111B, 3111D, 3112B, 3113B, 3114B are not approved in the 20th edition.

2a Only approved in 20th edition

3 "Methods for the Determination of Metals in Environmental Samples - Supplement I," EPA-600/R-94-111, May 1994. Available at NTIS, PB 94-184942.

4 Method 100.1, "Analytical Method for Determination of Asbestos Fibers in Water," EPA-600/4-83-043, EPA, September 1983. Available at NTIS, PB 83-260471.

5 Method 100.2, "Determination of Asbestos Structure Over 10-μm In Length in Drinking Water," EPA-600/R-94-134, June 1994. Available at NTIS, PB 94-201902.

6 Methods for the Determination of Organic and Inorganic Compounds in Drinking Water - Volume 1," document number EPA 815-R-00-014, August 2000.

7 "Methods for the Determination of Inorganic Substances in Environmental Samples," EPA-600/R-93-100, August 1993. Available at NTIS, PB94-121811.

8 Available from Books and Open-File Reports Section, U.S. Geological Survey, Federal Center, Box 25425, Denver, CO 80225-0425.

9 Industrial Method No. 129-71W, "Fluoride in Water and Wastewater," December 1972, and Method No. 380-75WE, "Fluoride in Water and Wastewater," February 1976, Technicon Industrial Systems, Tarrytown, NY 10591.

10 Methods 150.1, 150.2 and 245.2 are available from US EPA, NERL, Cincinnati, OH 45268. The identical methods were formerly in "Methods for Chemical Analysis of Water and Wastes," EPA-600/4-79-020, March 1983.

11 Method B-1011, "Waters Test Method for Determination of Nitrite/Nitrate in Water Using Single Column Ion Chromatography," Millipore Corporation, Waters Chromatography Division, 34 Maple Street, Milford, MA 01757.

12 Technical Bulletin 601 "Standard Method of Test for Nitrate in Drinking Water," July 1994, PN 221890-001, Thermo Orion, 500 Cummins Center, Beverly, MA 01915-9846. This method is identical to Orion WeWWG/5880, which is approved for nitrate analysis. ATI Orion republished the method in 1994, and renumbered it as 601, because the 1985 manual "Orion Guide to Water and Wastewater Analysis," which contained WeWWG/5880, is no longer available.

13 GLI Method 2, "Turbidity," November 2, 1992, GLI International, 9020 W Dean Rd. Milwaukee, Wisconsin 53224.

Table IV-3 Approved Methods for Primary Organic Chemicals [§141.24(e)]

Contaminant	Method[1] (Revision Number)
Benzene	502.2(2.1), 524.2(4.1)
Carbon tetrachloride	502.2(2.1), 524.2(4.1), 551.1(1.0)
Chlorobenzene	502.2(2.1), 524.2(4.1)
1,2-Dichlorobenzene	502.2(2.1), 524.2(4.1)
1,4-Dichlorobenzene	502.2(2.1), 524.2(4.1)
1,2-Dichloroethane	502.2(2.1), 524.2(4.1)
cis-1,2-Dichloroethylene	502.2(2.1), 524.2(4.1)
trans-1,2-Dichloroethylene	502.2(2.1), 524.2(4.1)
Dichloromethane	502.2(2.1), 524.2(4.1)
1,2-Dichloropropane	502.2(2.1), 524.2(4.1)
Ethylbenzene	502.2(2.1), 524.2(4.1)
Styrene	502.2(2.1), 524.2(4.1)
Tetrachloroethylene	502.2(2.1), 524.2(4.1), 551.1(1.0)
1,1,1-Trichloroethane	502.2(2.1), 524.2(4.1), 551.1(1.0)
Trichloroethylene	502.2(2.1), 524.2(4.1), 551.1(1.0)
Toluene	502.2(2.1), 524.2(4.1)
1,2,4-Trichlorobenzene	502.2(2.1), 524.2(4.1)
1,1-Dichloroethylene	502.2(2.1), 524.2(4.1)
1,1,2-Trichloroethane	502.2(2.1), 524.2(4.1), 551.1(1.0)
Vinyl chloride	502.2(2.1), 524.2(4.1)
Xylenes (total)	502.2(2.1), 524.2(4.1)
2,3,7,8-TCDD (dioxin)	1613
2,4-D (as acids, salts and esters)	515.1(4.0), 515.2(1.1), 515.3(1.0), 555(1.0), D5317-93, 515.4(1.0)
Alachlor	505(2.1)[1], 507(2.1), 508.1(2.0), 525.2(2.0), 551.1(1.0)
Atrazine	505(2.1)[1], 507(2.1), 508.1(2.0), 525.2(2.0), 551.1(1.0)
Benzo(a)pyrene	525.2(2.0), 550, 550.1
Carbofuran	531.1(3.1), 6610, 531.2(1.0)
Chlordane	505(2.1), 508(3.1), 508.1(2.0), 525.2(2.0)

Contaminant	Method[1] (Revision Number)
Dalapon	515.1(4.0), 515.3(1.0), 552.1(1.0), 552.2(1.0), 515.4(1.0)
Di(2-ethylhexyl)adipate	506(1.1), 525.2(2.0)
Di(2-ethylhexyl)phthalate	506(1.1), 525.2(2.0)
Dibromochloropropane (DBCP)	504.1(1.1), 551.1(1.0)
Dinoseb	515.1(4.0),515.2(1.1), 515.3(1.0), 555(1.0), 515.4(1.0)
Diquat	549.2(1.0)
Endothall	548.1(1.0)
Endrin	505(2.1), 508(3.1), 508.1(2.0), 525.2(2.0), 551.1(1.0)
Ethylene dibromide (EDB)	504.1(1.1), 551.1(1.0)
Glyphosate	547, 6651
Heptachlor	505(2.1), 508(3.1), 508.1(2.0), 525.2(2.0), 551.1(1.0)
Heptachlor Epoxide	505(2.1), 508(3.1), 508.1(2.0), 525.2(2.0), 551.1(1.0)
Hexachlorobenzene	505(2.1), 508(3.1), 508.1(2.0), 525.2(2.0), 551.1(1.0)
Hexachlorocyclopentadiene	505(2.1), 508(3.1), 508.1(2.0), 525.2(2.0), 551.1(1.0)
Lindane	505(2.1), 508(3.1), 508.1(2.0), 525.2(2.0), 551.1(1.0)
Methoxychlor	505(2.1), 508(3.1), 508.1(2.0), 525.2(2.0), 551.1(1.0)
Oxamyl	531.1(3.1), 6610, 531.2(1.0)
PCBs (as decachlorobiphenyl)[2] (as Aroclors)	508A(1.0) 505(2.1), 508(3.1), 508.1(2.0), 525.2(2.0)
Pentachlorophenol	515.1(4.0), 515.2(1.1), 515.3(1.0), 525.2(2.0), 555(1.0), D5317-93, 515.4(1.0)
Picloram	515.1(4.0), 515.2(1.1), 515.3(1.0), 555(1.0), D5317-93, 515.4(1.0)
Simazine	505(2.1), 507(2.1), 508.1(2.0), 525.2(2.0), 551.1(1.0)
2,4,5-TP (Silvex)	515.1(4.0), 515.2(1.1), 515.3(1.0), 555(1.0), D5317-93, 515.4(1.0)
Toxaphene	505(2.1), 508(3.1), 508.1(2.0), 525.2(2.0)
HAA5[4]	552.1(1.0), 552.2(1.0), SM6251
Total Trihalomethanes	502.2(2.1), 524.2(4.1), 551.1(1.0)

Footnotes

[1] Methods 508A, and 515.1 are in Methods for the Determination of Organic Compounds in Drinking Water, EPA-600/4-88-039, December 1988, Revised, July 1991. Methods 547, 550, and 550.1 are in Methods for the Determination

of Organic Compounds in Drinking Water - Supplement I, EPA-600-4-90-020, July 1990. Methods 515.2, 524.2, 548.1, 552.1 and 555 are in Methods for the Determination of Organic Compounds in Drinking Water - Supplement II, EPA-600/R-92-129. Methods 502.2, 504.1, 505, 506, 507, 508, 508.1, 515.1, 515.2, 524.2, 525.2, 531.1, 551.1, 552.2 are in Methods for the Determination of Organic Compounds in Drinking Water - Supplement III, EPA 600/R-95/131. Methods 513.3 and 549.2 are in Methods for the Determination of Organic and Inorganic Compounds in Drinking Water - Volume 1, EPA 815-R-00-014, August 2000. Method 1613, Tetra-Through Octa- Chlorinated Dioxins and Furans by Isotopic Dilution HRGC/HRMS, EPA-81/B-94-003, October 1994 These documents are available from the National Technical Information Service, NTIS PB91-231480, PB91-146027, PB92-207703, PB2000-106981 and PB95-104774, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161. The toll-free number is 800-553-6847. Method 1613 is available from USEPA Office of Water Resource Center (RC-4100), 401 M. Street S.W., Washington, D.C. 20460. The phone number is 202-260-7786. Method 6651 and 6610 are contained in the currently approved editions of *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, 1015 Fifteenth Street NW, Washington, D.C. 20005.

[2] PCBs are qualitatively identified as Aroclors and measured for compliance purposes as decachlorobiphenyl using Method 508A.

[3] A nitrogen-phosphorus detector should be substituted for the electron capture detector in Method 505 (or another approved method should be used) to determine alachlor, atrazine and simazine, if lower detection limits are required.

[4]The total of monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid and dibromoacetic acid.

Table IV-4 Approved Methods for Disinfectant Residuals

Public water systems need to measure residual disinfectant concentrations with one of the analytical methods in the following table. The methods are contained in the 18th, 19th and 20th editions of *Standard Methods for the Examination of Water and Wastewater.*

Residual[1]	Methodology	SM[3]
Free Chlorine[2]	Amperometric Titration	4500-Cl D
		D 1253-86
	DPD Ferrous Titrimetric	4500-Cl F
	DPD Colorimetric	4500-Cl G
	Syringaldahyde (FACTS)	4500-Cl H
Combined Chlorine (Chloramines)	Amperometric Titration	4500-Cl D
		D 1253-86
	DPD Ferrous Titrimetric	4500-Cl F
	DPD Colorimetric	4500-Cl G
Total Chlorine[2]	Amperometric Titration	4500-Cl D
		D 1253-86
	Amperometric Titration (low level measurement)	4500-Cl E
	DPD Ferrous Titrimetric	4500-Cl F
	DPD Colorimetric	4500-Cl G
	Iodometric Electrode	4500-Cl I
Chlorine Dioxide	Amperometric Titration	4500-ClO_2 C[4]
	DPD Method	4500-ClO_2 D
	Amperometric Titration	4500-ClO_2 E
Ozone	Indigo Method	4500-O_3 B

Footnotes

[1] If approved by the State, residual disinfectant concentrations for free chlorine and combined chlorine also may be measured by using DPD colorimetric test kits.

[2] Free and total chlorine residuals may be measured continuously by adapting a specified chlorine residual method for use with a continuous monitoring instrument provided the chemistry, accuracy, and precision of the measurement remain the same. Instruments used for continuous monitoring need to be calibrated with a grab sample measurement at least every five days, or with protocol approved by the State.

[3] *Standard Methods for the Examination of Water and Wastewater*, 18th, 19th or 20th Edition, American Public Health Association, 1015 Fifteenth Street NW, Washington, D.C. 20005.

[4] Method 4500-ClO_2 is not approved for determining compliance at 141.131(c) because the other two methods are superior.

Table IV-5 Recommended Methods for Secondary Drinking Water Contaminants

Analyses of aluminum, chloride, color, fluoride, foaming agents, iron, manganese, odor, silver, sulfate, total dissolved solids (TDS) and zinc to determine compliance under §143.3 may be conducted with the methods in the following table. Criteria for analyzing aluminum, iron, manganese, silver, and zinc samples with digestion or directly without digestion, and other mandatory procedures are contained in Section IV of "Technical Notes on Drinking Water Methods" EPA/600/R-94/173, October 1994. Measurement of pH may be conducted with one of the methods listed above in Section I under "Methods for Inorganic Chemicals."

Contaminant	EPA	ASTM[1]	SM[2]	Other
Aluminum	200.7[3]		3120B	
	200.8[3]		3113B	
	200.9[3]		3111D	
Chloride	300.0[4]	D4327-91	4110B	
		D512-89B	4500-Cl^-B,-D	
Color			2120B	
Fluoride	300.0	D4327-91 D1179-93	4110 B 4500-F^-B, C, D, E	380-75WE 129-71W[5]
Foaming Agents			5540C	
Iron	200.7[3]		3120B	
	200.9[3]		3111B	
			3113B	
Manganese	200.7[3]		3120B	
	200.8[3]		3111B	
	200.9[3]		3113B	
Odor			2150B	
Silver	200.7[3]		3120B	I-3720-85[6]
	200.8[3]		3111B	
	200.9[3]		3113B	
Sulfate	300.0[4]	D4327-91	4110B	
	375.2[4]	D516-90	4500-SO_4-E,-F	
			4500-SO_4-C,D	
TDS			2540C	
Zinc	200.7[3]		3120B	
	200.8[3]		3111B	

Footnotes

[1] *Annual Book of ASTM Standards*, Vols. 11.01 and 11.02, American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

[2] *Standard Methods for the Examination of Water and Wastewater*, 18th, 19th or 20th Edition, American Public Health Association, 1015 Fifteenth Street NW, Washington, D.C. 20005. Except 3111B, 3111D, 3112B, 3113B, 3114B are not approved in the 20th edition.

[3] "Methods for the Determination of Metals in Environmental Samples - Supplement I," EPA-600/R-94-111, May 1994. Available at NTIS, PB94-184942.

[4] "Methods for the Determination of Inorganic Substances in Environmental Samples," EPA-600/R-93-100, August 1993. Available at NTIS, PB94-121811.

[5] Industrial Method No. 129-71W, "Fluoride in Water and Wastewater," December 1972, and Method No. 380-75WE, "Fluoride in Water and Wastewater," February 1976, Bran and Lubbe, 1025 Busch Parkway Buffalo Grove, IL 60089. (Formerly Technicon Industrial Systems, Tarrytown, NY 10591)

[6] Available from Books and Open-File Reports Section, U.S. Geological Survey, Federal Center, Box 25425, Denver, CO 80225-0425.

Table IV-6 Sample Containers, Preservation and Holding Times for Regulated Parameters

Parameter/ Method	Preservative	Sample Holding Time	Extract Holding Time and Storage Conditions	Suggested Sample Size	Type of Container
Metals (except Hg)	HNO_3 pH<2	6 months		1 L	Plastic or Glass
Mercury	HNO_3 pH<2	28 days		100 mL	Plastic or Glass
Alkalinity	Cool, 4C	14 days		100 mL	Plastic or Glass
Asbestos	Cool, 4C	48 hours		1 L	Plastic or Glass
Chloride	none	28 days		100 mL	Plastic or Glass
Residual Disinfectant	none	immediately		200 mL	Plastic or Glass
Color	Cool, 4C	48 hours		100 mL	Plastic or Glass
Conductivity	Cool, 4C	28 days		100 mL	Plastic or Glass
Cyanide	Cool, 4C, Ascorbic acid (if chlorinated), NaOH pH>12	14 days		1 L	Plastic or Glass
Fluoride	none	1 month		100 mL	Plastic or Glass
Foaming Agents	Cool, 4C	48 hours			
Nitrate (chlorinated)	Cool, 4C non-acidified	14 days		100 mL	Plastic or Glass
Nitrate (non chlorinated)	Cool, 4C, non-acidified	48 hours		100 mL	Plastic or Glass
Nitrite	Cool, 4C	48 hours		100 mL	Plastic or Glass
Nitrate+ Nitrite	H2SO4 pH<2	28 days		100 mL	Plastic or Glass
Odor	Cool, 4C	24 hours		200 mL	Glass
pH	none	immediately		25 mL	Plastic or Glass
o-Phosphate	Cool, 4C	48 hours		100mL	Plastic or Glass

Parameter/ Method	Preservative	Sample Holding Time	Extract Holding Time and Storage Conditions	Suggested Sample Size	Type of Container
Silica	Cool, 4C	28 days		100 mL	Plastic
Solids (TDS)	Cool, 4C	7 days		100 mL	Plastic or Glass
Sulfate	Cool, 4C	28 days		100 mL	Plastic or Glass
Temperature	none	immediately		1 L	Plastic or Glass
Turbidity	Cool, 4C	48 hours		100 mL	Plastic or Glass
502.2	Sodium Thiosulfate or Ascorbic Acid, 4C, HCl pH<2	14 days		40-120 mL	Glass with PTFE Lined Septum
504.1	Sodium Thiosulfate Cool, 4C,	14 days	4C, 24 hours	40 mL	Glass with PTFE Lined Septum
505	Sodium Thiosulfate Cool, 4C	14 days (7 days for Heptachlor)	4C, 24 hours	40 mL	Glass with PTFE Lined Septum
506	Sodium Thiosulfate Cool, 4C, Dark	14 days	4C, dark 14 days	1 L	Amber Glass with PTFE Lined Cap
507	Sodium Thiosulfate Cool, 4C, Dark	14 days(see method for exceptions)	4C, dark 14 days	1 L	Amber Glass with PTFE Lined Cap
508	Sodium Thiosulfate Cool, 4C, Dark	7 days (see method for exceptions)	4C, dark 14 days	1 L	Glass with PTFE Lined Cap
508A	Cool, 4C	14 days	30 days	1 L	Amber Glass with PTFE Lined Cap
508.1	Sodium Sulfite HCl pH<2 Cool, 4C	14 days (see method for exceptions)	30 days	1 L	Glass with PTFE Lined Cap
515.1	Sodium Thiosulfate Cool, 4C, Dark	14 days	4C, dark 28 days	1 L	Amber Glass with PTFE Lined Cap

Parameter/ Method	Preservative	Sample Holding Time	Extract Holding Time and Storage Conditions	Suggested Sample Size	Type of Container
515.2	Sodium Thiosulfate or Sodium Sulfite HCl pH<2 Cool, 4C, Dark	14 days	≤4C, dark 14 days	1 L	Amber Glass with PTFE Lined Cap
515.3	Sodium Thiosulfate Cool, 4C, Dark	14 days	≤4C, dark 14 days	50 mL	Amber Glass with PTFE Lined Cap
515.4	Sodium Sulfite, dark, cool ≤10C fro first 48 hr. ≤6C thereafter	14 days	≤0C 21 days	40 mL	Amber glass with PTFE lined septum
524.2	Ascorbic Acid or Sodium Thiosulfate HCl pH<2, Cool 4C	14 days		40-120 mL	Glass with PTFE Lined Septum
525.2	Sodium Sulfite, Dark, Cool, 4C, HCl pH<2	14 days (see method for exceptions)	≤4C 30 days	1 L	Amber Glass with PTFE Lined Cap
531.1, 6610	Sodium Thiosulfate, Monochloroacetic acid, pH<3, Cool, 4C	Cool 4C 28 days		60 mL	Glass with PTFE Lined Septum
531.2	Sodium Thiosulfate, Potassium Dihydrogen Citrate buffer to pH 4, dark, ≤10C for first 48 hr, ≤6C thereafter	28 days		40 mL	Glass with PTFE Lined Septum
547	Sodium Thiosulfate Cool, 4C	14 days(18 mo.frozen)		60 mL	Glass with PTFE Lined Septum

Parameter/ Method	Preservative	Sample Holding Time	Extract Holding Time and Storage Conditions	Suggested Sample Size	Type of Container
548.1	Sodium Thiosulfate (HCl pH 1.5-2 if high biological activity) Cool, 4C, Dark	7 days	≤4C 14 days	≥ 250 mL	Amber Glass with PTFE Lined Septum
549.2	Sodium Thiosulfate, (H_2SO_4 pH<2 if biologically active) Cool, 4C, Dark	7 days	21 days	≥ 250mL	High Density Amber Plastic or Silanized Amber Glass
550, 550.1	Sodium Thiosulfate Cool, 4C, HCl pH<2	7 days	4C, Dark 550, 30 days 550.1, 40 days	1 L	Amber Glass with PTFE Lined Cap
551.1	Sodium Sulfite, Ammonium Chloride, pH 4.5-5.0 with phosphate buffer Cool, 4C	14 days		≥ 40 mL	Glass with PTFE Lined Septum
552.1	Ammonium chloride Cool, 4C, Dark	28 days	≤4C, dark 48 hours	250 mL	Amber Glass with PTFE Lined Cap
552.2	Ammonium chloride Cool, 4C, Dark	14 days	≤4C, dark 7 days ≤-10C 14 days	50mL	Amber Glass with PTFE Lined Cap
555	Sodium Sulfite HCl, pH≤2 Dark, Cool 4C	14 days		≥ 100 mL	Glass with PTFE Lined cap
1613	Sodium Thiosulfate Cool, 0-4C, Dark		Recommend 40 days	1 L	Amber Glass with PTFE Lined Cap

Table IV-7 MCLs and Detection Limits Requirements in the CFR (mg/L) to Composite

Inorganics	MCL*	MCLG	Detection Limit Required to Composite [§141.23(a)(4)]
Asbestos	7 MFL	7 MFL	1.4 MFL
Bromate	0.010		NA
Chlorite	1.0		NA
Cyanide	0.2	0.2	0.04
Fluoride	4.0		0.8
Nitrate	10	10	2
Nitrite	1	1	0.2

* The monitoring trigger for the inorganics is the MCL except for both nitrate and nitrite, which are ½ the MCL

Metals	MCL *	MCLG	Detection Limit Required to Composite [§141.23(a)(4)]
Antimony	0.006	0.006	0.001
Arsenic	0.01	-	0.01
Barium	2	2	0.4
Beryllium	0.004	0.004	0.0008
Cadmium	0.005	0.005	0.001
Chromium	0.1	0.1	0.02
Copper**	1.3	1.3	0.001 0.02 (for direct aspiration AA)
Lead**	0.015	zero	0.001
Mercury	0.002	0.002	0.0004
Selenium	0.05	0.05	0.01
Thallium	0.002	0.0005	0.0004

* The monitoring trigger for metals is the MCL unless compositing, then 1/5 MCL is required
**Action Level

TABLE IV-8 VOC MCLs and Detection Limit Requirements in the CFR (mg/L) for Compliance Monitoring

Volatile Organics*	MCL	MCLG	Required MDL
THMs	0.08	NA	NA
HAA5	0.06	NA	NA
Benzene	0.005	zero	0.0005
Carbon tetrachloride	0.005	zero	0.0005
Chlorobenzene	0.1	0.1	0.0005
o-Dichlorobenzene	0.6	0.6	0.0005
p-Dichlorobenzene	0.075	0.075	0.0005
1,2-Dichloroethane	0.005	zero	0.0005
1,1-Dichloroethylene	0.007	0.007	0.0005
c-1,2-Dichloroethylene	0.07	0.07	0.0005
t-1,2-Dichloroethylene	0.1	0.1	0.0005
Dichloromethane	0.005	zero	0.0005
1,2-Dichloropropane	0.005	zero	0.0005
Ethylbenzene	0.7	0.7	0.0005
Styrene	0.1	0.1	0.0005
Tetrachloroethylene	0.005	zero	0.0005
Toluene	1	1	0.0005
1,2,4-Trichlorobenzene	0.07	0.07	0.0005
1,1,1-Trichloroethane	0.2	0.2	0.0005
1,1,2-Trichloroethane	0.005	0.003	0.0005
Trichloroethylene	0.005	zero	0.0005
Vinyl chloride	0.002	zero	0.0005
Xylenes	10	10	0.0005

*A laboratory must be able to achieve an MDL of 0.0005 mg/L to be certified to analyze samples for compliance monitoring [§141.24(f)(17)(i)(E) and (ii)(C)]. This is also the monitoring trigger for VOCs [§141.24(f)(11)].

TABLE IV-9 SOC MCLs and Detection Limit Requirements in the CFR to Reduce Monitoring (mg/L)

SOCs	MCL	MCLG	Monitoring Trigger*
Alachlor	0.002	zero	0.0002
Atrazine	0.003	0.003	0.0001
Benzo(a)pyrene	0.0002	zero	0.00002
Carbofuran	0.04	0.04	0.0009
Chlordane	0.002	zero	0.0002
2,4-D	0.07	0.07	0.0001
Di(2-ethylhexyl)adipate	0.4	0.4	0.0006
Di(2-ethylhexyl)phthalate	0.006	zero	0.0006
Dibromochloropropane (DBCP)	0.0002	zero	0.00002
Dalapon	0.2	0.2	0.001
Dinoseb	0.007	0.007	0.0002
Dioxin (2,3,7,8-TCDD)	$3x10^{-8}$	zero	$5x10^{-9}$
Diquat	0.02	0.02	0.0004
Endothall	0.1	0.1	0.009
Endrin	0.002	0.002	0.00001
Ethylenedibromide (EDB)	0.00005	zero	0.00001
Glyphosate	0.7	0.7	0.006
Heptachlor	0.0004	zero	0.00004
Heptachlor Epoxide	0.0002	zero	0.00002
Hexachlorobenzene	0.001	zero	0.0001
Hexachlorocyclopentadiene	0.05	0.05	0.0001
Lindane	0.0002	0.0002	0.00002
Methoxychlor	0.04	0.04	0.0001
Oxamyl	0.2	0.2	0.002
PCBs (as decachlorobiphenyl)	0.0005	zero	0.0001
Pentachlorophenol	0.001	zero	0.00004
Picloram	0.5	0.5	0.0001
Simazine	0.004	0.004	0.00007
Toxaphene	0.003	zero	0.001
2,4,5-TP (Silvex)	0.05	0.05	0.0002

*The monitoring triggers for SOCs listed in the regulation are also required for compositing but are not required by regulation for certification [§141.24(g)(7),(10)(i) and (18)].

Table IV-10 MCL and Proficiency Testing Sample Acceptance Criteria in the CFR Primary and Secondary Drinking Water Regulations [§141.23(k)(3)(ii) and 141.24(f)(17) and (19)]

Regulated Parameter	MCL/ [SMCL]	MCLG	Acceptance
METALS			
Aluminum	[0.05-0.2]	-	
Antimony	0.006	0.006	± 30%
Arsenic	0.01	-	
Barium	2.0	2.0	± 15%
Beryllium	0.004	0.004	± 15%
Cadmium	0.005	0.005	± 20%
Calcium	-	-	
Chromium	0.1	0.1	± 15%
Copper	1.3/90% [1.0]	1.3	± 10%
Iron	[0.3]	-	
Lead	0.015/90%	zero	± 30%
Manganese	[0.05]	-	
Mercury	0.002	0.002	± 30%
Selenium	0.05	0.05	± 20%
Silica	-	-	
Silver	[0.10]		
Sodium	20[1]	-	
Thallium	0.002	0.0005	± 30%
Zinc	[5.0]	-	

[1] Recommended Level

Regulated Parameter	MCL/ [SMCL]	MCLG	Acceptance
INORGANICS			
Alkalinity	-	-	
Asbestos	7MF/L>10u	7MF/L>10u	2 Std Dev
Bromate	0.010		95% conf interval around mean
Chloride	[250]	-	
Chlorite	1.0		95% conf interval around mean
Residual Disinfectant	detectable	-	
Color	[15cu]	-	
Conductivity	-	-	
Corrosivity	[non-corrosive]	-	
Cyanide	0.2	0.2	$\pm$ 25%
Fluoride	4.0 [2.0]	-	$\pm$ 10%
Foaming Agents	[0.5]	-	
Nitrate (as N)	10	10	$\pm$ 10%
Nitrite (as N)	1	1	$\pm$ 15%
Nitrate/Nitrite (as N)	10	10	
Odor	[3 t.o.n.]	-	
pH	6.5-8.5 [6.5-8.5]	-	
o-Phosphate	-	-	
Solids(TDS)	[500]	-	
Sulfate	deferred [250]	deferred	
Temperature	-	-	

Regulated Parameter	MCL/ [SMCL]	MCLG	Acceptance
VOLATILES			
Trihalomethanes(Total)	0.080		95% conf interval around mean
HAA5	0.060		95% conf interval around mean
Benzene	0.005	zero	*
Carbon tetrachloride	0.005	zero	*
Chlorobenzene	0.1	0.1	*
p-Dichlorobenzene	0.075 [0.005]	0.075	*
o-Dichlorobenzene	0.6	0.6	*
1,2-Dichloroethane	0.005	zero	*
1,1-Dichloroethylene	0.007	0.007	*
c-1,2-Dichloroethylene	0.07	0.07	*
t-1,2-Dichloroethylene	0.1	0.1	*
Dichloromethane	0.005	zero	*
1,2-Dichloropropane	0.005	zero	*
Ethylbenzene	0.7	0.7	*
Styrene	0.1	0.1	*
Tetrachloroethylene	0.005	zero	*
Toluene	1	1	*
1,2,4-Trichlorobenzene	0.07	0.07	*
1,1,1-Trichloroethane	0.2	0.2	*
1,1,2-Trichloroethane	0.005	0.003	*
Trichloroethylene	0.005	zero	*
Vinyl chloride	0.002	zero	± 40%
Xylenes(Total)	10	10	*

Regulated Parameter	MCL/ [SMCL]	MCLG	Acceptance
SYNTHETIC ORGANICS			
Alachlor	0.002	zero	± 45%
Aldicarb	Postponed	Postponed	2 Std Dev
Aldicarb Sulfoxide	Postponed	Postponed	2 Std Dev
Aldicarb Sulfone	Postponed	Postponed	2 Std Dev
Atrazine	0.003	0.003	± 45%
Carbofuran	0.04	0.04	± 45%
Chlordane	0.002	zero	± 45%
2,4-D	0.07	0.07	± 50%
Dalapon	0.2	0.2	2 Std Dev
Dibromochloropropane(DBCP)	0.0002	zero	± 40%
Dinoseb	0.007	0.007	2 Std Dev
Diquat	0.02	0.02	2 Std Dev
Endothall	0.1	0.1	2 Std Dev
Endrin	0.002	0.002	± 30%
Ethylenedibromide(EDB)	0.00005	zero	± 40%
Glyphosate	0.7	0.7	2 Std Dev
Heptachlor	0.0004	zero	± 45%
Heptachlor epoxide	0.0002	zero	± 45%
Lindane	0.0002	0.0002	± 45%
Methoxychlor	0.04	0.04	± 45%
Oxamyl (Vydate)	0.2	0.2	2 Std Dev
Pentachlorophenol	0.001	zero	± 50%

Regulated Parameter	MCL/ [SMCL]	MCLG	Acceptance
Picloram	0.5	0.5	2 Std Dev
Simazine	0.004	0.004	2 Std Dev
Toxaphene	0.003	zero	± 45%
2,4,5-TP(Silvex)	0.05	0.05	± 50%
Hexachlorobenzene	0.001	zero	2 Std Dev
Hexachlorocyclopentadiene	0.05	0.05	2 Std Dev
Benzo(a)pyrene	0.0002	zero	2 Std Dev
PCBs (as decachlorobiphenyl)	0.0005	zero	0-200%
2,3,7,8-TCDD(Dioxin)	$3x10^{-8}$	zero	2 Std Dev
Acrylamide	Treatment	zero	NA
Epichlorohydrin	Treatment	zero	NA
Di(2-ethylhexyl)adipate	0.4	0.4	2 Std Dev
Di(2-ethylhexyl)phthalate	0.006	zero	2 Std Dev

* the acceptance limits for VOCs are ±20% at ≥0.010mg/L and ±40% at <0.010mg/L
NA - Not Applicable

Table IV-11 Promulgated Organic Drinking Water Methods (As of January 2005)

Method Number	Method Title	Revision
502.2[a]	Volatile Organic Compounds in Water By Purge and Trap Capillary Column Gas Chromatography with Photoionizatio and Electrolytic Conductivity Detectors in Series	2.1
504.1[a]	1,2-Dibromoethane (EDB), 1,2-Dibromo-3-Chloropropane (DBCP), and 1,2,3-Trichloro-propane (123TCP) in Water by Microextraction and Gas Chromatography	1.1
505[a]	Analysis of Organohalide Pesticides and Commercial Polychlorinated Biphenyl Products in Water by Micro-Extraction and Gas Chromatography	2.1
506[a]	Determination of Phthalate and Adipate Esters in Drinking Water by Liquid-Liquid Extraction or Liquid-Solid Extraction and Gas Chromatography with Photoionization Detection	1.1
507[a]	Determination of Nitrogen and Phosphorus-Containing Pesticides in Water by Gas Chromatography with a Nitrogen-Phosphorus Detector	2.1
508[a]	Determination of Chlorinated Pesticides in Water by Gas Chromatography with An Electron Capture Detector	3.1
508A[b]	Screening for Polychlorinated Biphenyls by Perchlorination and Gas Chromatography	1.0
508.1[a]	Determination of Chlorinated Pesticides, Herbicides, and Organohalides by Liquid-Solid Extraction and Electron Capture Gas Chromatography	2.0
515.1[b]	Determination of Chlorinated Acids in Water by Gas Chromatography with an Electron Capture Detector	4.0
515.2[a]	Determination of Chlorinated Acids in Water Using Liquid-Solid Extraction and Gas Chromatography With an Electron Capture Detector	1.1
515.3[e]	Determination of Chlorinated Acids in Drinking Water by Liquid-Liquid Extraction, Derivatization and Gas Chromatography with Electron Capture Detection	1.0
515.4[f]	Determination of Chlorinated Acids in Drinking Water by Liquid-Liquid Microextraction, Derivatization and Fast Gas Chromatography with Electron Capture Detection	1.0
524.2[a]	Measurement of Purgeable Organic Compounds in Water by Capillary Column Gas Chromatography/Mass Spectrometry	4.1
525.2[(a)]	Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry	2.0
531.1[a]	Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC with Post Column Derivatization	3.1

Table IV-11 Promulgated Organic Drinking Water Methods (As of January 2005)

Method Number	Method Title	Revision
531.2[f]	Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC with Post Column Derivatization	1.0
547[c]	Determination of Glyphosate in Drinking Water By Direct-Aqueous-Injection HPLC, Post-Column Derivatization, and Fluorescence Detection	
548.1[d]	Determination of Endothall in Drinking Water by Ion Exchange Extraction, Acidic Methanol Methylation Gas Chromatography/Mass Spec.	1.0
549.2[e]	Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection	1.0
550[c]	Determination of Polycyclic Aromatic Hydro-carbons in Drinking Water by Liquid-Liquid Extraction and HPLC with Coupled Ultraviolet and Fluorescence Detection	
550.1[c]	Determination of Polycyclic Aromatic Hydro-carbons in Drinking Water by Liquid-Solid Extraction and HPLC with Coupled Ultraviolet and Fluorescence Detection	
551.1[a]	Determination of Chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogenated Pesticides/Herbicides in Drinking Water by Liquid-Liquid Extraction and Gas Chromatography with Electron-Capture Detection	1.0
552.1[d]	Determination of Haloacetic Acids and Dalapon in Drinking Water by Ion Exchange Liquid-Solid Extraction and Gas Chromatography With Electron Capture Detection	1.0
552.2[a]	Determination of Haloacetic Acids and Dalapon in Drinking Water by Liquid-Liquid Extraction, Derivatization and Gas Chromatography With Electron Capture Detection	1.0
555[d]	Determination of Chlorinated Acids in Water by High Performance Liquid Chromatography with a Photodiode Array Ultraviolet Detector	1.0

Footnotes

[a]These methods are in the manual titled "Methods for the Determination of Organic Compounds in Drinking Water - Supplement III," document number EPA/600/R-95/131, August 1995. This manual is available from the National Technical Information Service (NTIS*). Address your request to NTIS and ask for their order number PB95-261616, cost is $101.00

[b]These methods are in the manual titled "Methods for the Determination of Organic Compounds in Drinking Water," document number EPA/600/4-88/039, December 1988 (Revised July 1991). This manual is available from the National Technical Information Service (NTIS*). Address your request to NTIS and ask for their order number PB91-231480; cost is $77.50.

[c]These methods are in the manual titled "Methods for the Determination of Organic Compounds in Drinking Water - Supplement I," document number EPA/600/4-90/020, July 1990. This manual is available from the National Technical Information Service (NTIS*). Address your request to NTIS and ask for their order number PB91-146027; cost is $58.50.

[d]These methods are in the manual titled "Methods for the Determination of Organic Compounds in Drinking Water Supplement II," document number EPA/600/R-92/129, August 1992. This manual is available from the National Technical Information Service (NTIS*). Address your request to NTIS and ask for their order number PB92-207703; cost is $63.00.

[e]These methods are in the manual titled, "Methods for the Determination of Organic and Inorganic Compounds in Drinking Water - Volume 1," document number EPA 815-R-00-014, August 2000. This manual is available from the National Technical Information Service (NTIS*). Address your request to NTIS and ask for their order number PB2000-106981; cost is $71.50.

[f]Stand alone methods. These manuals are available at the EPA web site at:
http://www.epa.gov/ogwdw/methods/sourcalt.html

*National Technical Information Service (NTIS)
5285 Port Royal Road
Springfield, VA 22161
Phone number: 800-553-6847
Fax number: 703-0605-6900
There is a $5.00 handling charge for the total purchase.

**Microbiological and Chemical Exposure Assessment Research Division,
Chemical Exposure Research Branch, Room 564
U. S. Environmental Protection Agency
26 West Martin Luther King Drive
Cincinnati, OH 45268
Phone number: 513-569-7586
Fax number: 513-569-7757
E-mail address: dwmethods-help@epamail.epa.gov

Example Checklists for On-Site Evaluation of Laboratories Analyzing Drinking Water

General Audit Information

Laboratory	
Street	
City, State	
Zip	
Telephone No. Fax No.	
Audit Team Leader	
Audit Team Members	
Audit Team Affiliation	
Date	

Laboratory _____ **Evaluator** _____

Location _____ **Date** _____

PHYSICAL FACILITY

Item	Acceptable Yes	Acceptable No	Comments
Environment			
Heating/Cooling/Humidity			
Lighting			
Ventilation/Exhaust hoods			
Cleanliness			
Electrical and water services			
Work Space			
Separation of incompatible testing areas			
Controlled access where appropriate			
Housekeeping			
Unencumbered access			
Adequate work space			
Storage			
Chemicals properly stored and dated			
Standards properly stored, dated and labeled with concentration, preparer's name and solvent, origin, purity & traceability			
Computers & automated equipment			
Safety procedures			

Laboratory _____ **Evaluator** _____

Location _____ **Date** _____

PERSONNEL (Use additional paper if necessary.)

Position/ Title	Name	Education Level Degree/Major*	Specialized Training	Present Specialty	Experience
Laboratory Director					
Manager					
Supervisors					
Instrument Operators					
AA					
TEM					
HPLC					
GC					
ICP					
GC/MS					
IC					
Other analysts					

	Yes	No	Comments
An organization chart available			
QA manager has line authority			
Personnel job descriptions and resumes available			
Personnel training documented			

*If the major is not in chemistry, list hours of college level courses in chemistry.

Laboratory _____ **Evaluator** _____

Location _____ **Date** _____

QUALITY ASSURANCE AND DATA REPORTING

Item	Comments	Satisfactory Yes	Satisfactory No
QA plan			
Organization			
Sampling SOPs available and used Preservation Containers Holding times Samplers trained			
Sample Rejection			
Laboratory sample handling Log in procedure Bound log book or secure computer log in Storage Tracking			
Analytical Methods Written methods available Approved methods used SOPs available and used			
Calibration Type and frequency Source of standards Data comparability Instrument tuning			
Blanks Trip Field Method			
Method Detection Limits Initial Frequency Acceptability			
Precision and Accuracy Initial Frequency Acceptability Control charts Laboratory fortified blanks Matrix duplicates			

Item	Comments	Satisfactory Yes	Satisfactory No
Other QC Checks Performance check samples Internal and surrogate standards Matrix spikes and replicates			
Qualitative Identification/ Confirmation			
Performance Evaluation Samples Analyzed			
Data Reduction and Validation Calculations Transcription Significant Figures Validation			
Preventive Maintenance			
Records Retention			
Corrective Action			

Laboratory _____ **Evaluator** _____

Location _____ **Date** _____

Item	No. of Units	Method	Manufacturer	Model	Satisfactory Yes	Satisfactory No
Analytical Balance 0.1 mg readability Stable base ASTM type 1 or 2 weights Service contracts						
Magnetic Stirrer Variable speed, TFE coated stir bar						
pH Meter Accuracy ±0.1 units Line or battery Usable with specific ion electrodes						
Conductivity Meter Readable in ohms or mhos Range of 2 ohms to 2 mhos Line or battery						
Hot Plate - temp control						
Centrifuge To 3000 rpm, Option of 4 x 50 mL						
Color Standards To verify wavelengths photometers Should cover 200-800 nm						
Refrigerator/Freezer Standard laboratory, explosion proof for organics Capable of maintaining nominal temperature of 4C						

Item	No. of Units	Method	Manufacturer	Model	Satisfactory Yes	Satisfactory No
Drying Oven Gravity or convection Controlled from room temp to 180°C or higher(±2°C)						
Muffle Furnace To 450°C for cleaning organic glassware						
Thermometer Mercury filled Celsius 1°C or finer subdivision to 180°C NIST Certified or traceable						
Glassware Borosilicate Volumetrics should be Class A						
Spectrophotometer Range 400 - 700 nm Band width - < 20 nm Use several size & shape cells Path length 1 - 5 cm		Cyanide, Fluoride Mercury Nitrate/Nitrite				
Filter Photometer Range 400 - 700 nm Band width 10 -70 nm Use several size & shape cells Pathlength 1 - 5 cm		same as above				
Amperometric Titrator		Disinfectants				
Specific Ion Meter Accuracy ± 1 mV		Cyanide Fluoride Nitrate				

Item	No. of Units	Method	Manufacturer	Model	Satisfactory Yes	Satisfactory No
Inductively Coupled Plasma (sequential, simultaneous) Computer controlled Background correction Radio frequency generator Argon gas supply Mass Spectrometer Range 5-250 amu Resolution 1 amu peak width at 5% peak height		200.7, 3120B 200.8				
Water Bath Electric or steam heat Controllable within 5°C to 100°C		Mercury Nitrate Pesticides				
Ion Chromatography Conductivity detector, UV detector Suppressor column, Separator column		Fluoride, Chloride Nitrate/Nitrite Bromate, Chlorite				
Atomic Absorption Spectrophotometer Single channel, Single or double beam Grating monochrometer Photo multiplier detector Adjustable slits, Range 190-800 nm Readout system: Response time compatible with AA Able to detect positive interference for furnace Chart recorder, CRT or hard copy printer		Metals				
Air/Acetylene commercial grade		Barium, Copper				

Item	No. of Units	Method	Manufacturer	Model	Satisfactory Yes	No
Nitrous Oxide - comm. grade		Barium				
Graphite Furnace Argon or Nitrogen (commercial grade) Reach required temperatures Background corrector provision for offline analysis Pipets and tips microliter capacity with disposable tips 5-100 microliters metal free tips		Antimony, Lead Arsenic, Barium Beryllium Cadmium, Nickel Chromium Selenium Thallium Copper				
Arsine Generator		Arsenic, Selenium				
Hydride Generator hydrogen, commercial grade		Antimony Arsenic, Selenium				
Mercury Analyzer Spectrophotometer Dedicated analyzer having a mercury lamp acceptable Adsorption cell: 10 cm quartz cell with quartz end windows or 11.5 cm plexiglass cell with 2.5 cm ID Air pump to deliver flow of at least 1 L/min Aeration tube with coarse glass frit Flowmeter to measure air flow of 1 L/min Drying unit: 6 in. tube with 20 grams magnesium perchlorate or heating device or lamp to prevent condensation on cell		Mercury				

Item	No. of Units	Method	Manufacturer	Model	Satisfactory Yes	Satisfactory No
Glassware Separatory funnels Kuderna Danish (K-D) concentrators		SOCs				
Gas Chromatography Split/splitless injection Oven temp. control ± 0.2°C Recorder, hard copy Oven temp. programmer Sub-ambient accessory Variable-constant differential flow control		Organics				
Electron Capture detector Linearized Radiological Check		504.1, 505 508, 508.1 508A, 515.1 515.2, 551, 552.1				
Electrolytic Conductivity/Photoionization detector		502.2 506 (PID only)				
Nitrogen Phosphorus detector		507				
Mass spectrometer (quadrupole or ion trap) All glass enrichment device All glass transfer line Electron ionization at ≥70 eV Scanning 35-260 amu ≤2 sec Interfaced data system		524.2, 525.2 548.1				
Purge & Trap system All glass purger 5/25 mL sample size		502.2, 524.2				

Item	No. of Units	Method	Manufacturer	Model	Satisfactory Yes	Satisfactory No
High Performance Liquid Chromatography Constant flow Capable of injecting 20-500 µL						
Gradient system post-column reactor fluorescence detector Absorption at 340nm and 308nm		531.1, 6610				
Gradient system UV detector at 254 nm fluorescence detector excitation at 280 nm detection at > 389 nm		550, 550.1				
Isocratic system photodiode array detector excitation at 257 nm detection at >308 nm		549.2				
Isocratic system post-column reactor fluorescence detector excitation at 340 nm detection at >455 nm		547				
Gradient system photodiode array/UV detector 210-310 nm		555				

Item	No. of Units	Method	Manufacturer	Model	Satisfactory Yes	No
Auto Analysis System multi-channel pump manifold, colorimeter		Cyanide, Nitrate/nitrite				
Transmission Electron Microscope 80 kV 300-100,000X magnification 1 nm resolution calibrate screen SAED and ED		Asbestos				

Laboratory _____ **Evaluator** _____

Location _____ **Date** _____

METHODOLOGY

Contaminant	**Method(s)** Name/Number and revision	**Reference** Cite source, year, page	**Samples/Mo**	**Satisfactory** Yes	No
Antimony					
Arsenic					
Barium					
Beryllium					
Cadmium					
Chromium					
Copper					
Lead					
Mercury					
Selenium					
Thallium					
Asbestos					
Cyanide					
Bromate					
Chlorite					
Fluoride					

Contaminant	Method(s) Name/Number and revision	Reference Cite source, year, page	Samples/Mo	Satisfactory Yes	No
Nitrate					
Nitrite					
Total THMs					
HAA5					
VOCs					
Herbicides					
Pesticides					
EDB/DBCP					
Dioxin					
Other SOCs					
PCBs					
Carbamates					
Diquat					
Endothall					
Glyphosate					

Laboratory _____ **Evaluator** _____

Location _____ **Date** _____

SAMPLE COLLECTION

Item	Comments	Satisfactory Yes	Satisfactory No
Trained Sample Collector			
Representative sampling			
Complete sample form			
Appropriate sampling and preservation			
Samples exceeding holding times discarded			
VOCs & THMs Hermetic seal			

Laboratory _____ **Evaluator** _____

Location _____ **Date** _____

SAMPLE HANDLING AND PRESERVATION

Contaminant	**Container** material & size	**Preservatives**	**Holding Time** Sample	Extract	**Satisfactory** Yes	No
Mercury						
Metals						
Asbestos						
Cyanide						
Fluoride						
Nitrate						
Nitrite						
Bromate						
Clorite						
Total THMs						
HAA5						
VOCs						
Herbicides						
Pesticides						
EDB/DBCP						

Contaminant	Container material & size	Preservatives	Holding Time Sample	Extract	Satisfactory Yes	No
Dioxin						
Other SOCs						
Carbamates						
Diquat						
Endothall						
Glyphosate						

Chapter V
Critical Elements for Microbiology

Note 1: This chapter uses the term "must" to refer to certification criteria that are required by the National Primary Drinking Water Regulations. The term "should" is used for procedures that, while not specifically required by the regulations, are considered good laboratory practices. To assure the validity of the data, it is critical that laboratories observe both the regulatory and non-regulatory criteria. Certification Officers have the prerogative to refuse certification if the quality control data are judged unsatisfactory or insufficient.

Note 2: Quality control items, designated by a "QC," necessitate written records. Each record should include analyst's initials and date(s).

Note 3: References to *Standard Methods for the Examination of Water and Wastewater* are to the 18th, 19th, and 20th editions (except where specifically noted).

1. Personnel

1.1 Supervisor/Consultant

The supervisor of the microbiology laboratory should have a bachelor's degree in microbiology, biology, or equivalent. Supervisors who have a degree in a subject other than microbiology should have had at least one college-level microbiology laboratory course in which environmental microbiology was covered. In addition, the supervisor should have a minimum of two weeks training at a Federal agency, State agency, or academic institution in microbiological analysis of drinking water or 80 hours of on-the-job training in water microbiology at a certified laboratory, or other training acceptable to the State or EPA. If a supervisor is not available (and a waiver not granted per paragraph 1.3), a consultant having the same qualifications may be substituted, as long as the laboratory can document that the consultant is acceptable to the State and is present on-site frequently enough to satisfactorily perform a supervisor's duties.

The laboratory supervisor has the responsibility to ensure that all laboratory personnel have demonstrated their ability to satisfactorily perform the analyses to which they are assigned and that all data reported by the laboratory meet the required quality assurance and regulatory criteria.

1.2 Analyst (or equivalent job title)

The analyst should perform microbiological tests with minimal supervision and have at least a high school education. In addition, the analyst should have a minimum of at least three months of bench experience in water, milk, or food microbiology. The analyst should also have training acceptable to the State (or EPA for non-primacy States) in microbiological analysis of drinking water and a minimum of 30 days of on-the-job training in drinking water microbiology under an experienced analyst. Analysts should take advantage of workshops and training programs that may be available from State regulatory agencies, professional societies, and manufacturers. Before analyzing compliance samples, the analyst should demonstrate acceptable results on unknown samples.

1.3 Waiver of Academic Training

The certification authority may waive the need for the above specified academic training, on a case-by-case basis, for highly experienced analysts. The certification authority may also waive the need for the above specified training, on a case-by-case basis, for supervisors of laboratories associated with drinking water systems that only analyze samples from that system. If such a waiver for supervisor training is granted, the certification authority will prepare a written and signed justification for such a waiver and have it available for inspection. Laboratories should also keep a copy of the waiver.

1.4 Personnel Records

Personnel records that include academic background, specialized training courses completed, and types of microbiological analyses conducted should be maintained on laboratory analysts.

2. Laboratory Facilities

Laboratory facilities should be clean, temperature and humidity controlled, and have adequate lighting at bench tops. The

laboratory should maintain effective separation between areas where activities are incompatible, minimize traffic flow and ensure that contamination does not adversely affect data quality. Bench tops and floors should be of a material that is easily cleaned and disinfected. Laboratory facilities should have sufficient bench-top area for processing samples; storage space for media, glassware, and portable equipment; floor space for stationary equipment (incubators, water baths, refrigerators, etc.); and associated area(s) for cleaning glassware and sterilizing materials. They should also have provisions for disposal of microbiological waste.

3. Laboratory Equipment and Supplies

The laboratory must have the equipment and supplies needed to perform the approved methods for which certification has been requested.

3.1 pH Meter

3.1.1 Accuracy and scale graduations should be within ±0.1 units.

3.1.2 pH buffer aliquots should be used only once.

3.1.3 Electrodes should be maintained according to the manufacturer's recommendations.

QC **3.1.4** pH meters should be standardized before each use period with pH 7.0 and either pH 4.0 or 10.0 standard buffers, whichever range covers the desired pH of the media or reagent. The date and buffers used should be recorded in a logbook, along with analyst's initials.

QC **3.1.5** Record pH meter slope monthly, after calibration.

3.1.5.1 If the pH meter does not have a feature to automatically calculate the slope, but can provide the pH in millivolts (mV), use the following formula to calculate the slope.

Slope (as %) = |mV at pH 7 - mV at pH 4| x 100/177

3.1.5.2 If the slope is below 95% or above 105%, the electrode or meter may need maintenance. Follow manufacturer's instructions for electrode maintenance and general cleaning.

QC **3.1.6** Commercial buffer solution containers should be dated upon receipt and when opened. Buffers should be discarded by the expiration date.

3.2 *Balance (top loader or pan)*

3.2.1 Balances should have readability of 0.1 g.

3.2.2 Balances should provide a sensitivity of at least 0.1 g for a load of 150 g, and 1 mg for a load of 10 g or less.

QC **3.2.3** Balances should be calibrated monthly using ASTM Class 1, 2, or 3 weights (minimum of three traceable weights which bracket laboratory weighing needs, with a readability of 0.1 g.)(ASTM, 1916 Race St., Philadelphia, PA 19103). Non-reference weights should be calibrated every six months with reference weights. Record calibrations in a logbook with the initials of the individual performing the calibration. Correction values should be on file and used. A reference weight should be re-certified every five years. Damaged or corroded weights should be replaced.

QC **3.2.4** Service contracts or internal maintenance protocols and maintenance records should be available. Maintenance, calibration, and cleaning should be conducted at least annually by a qualified independent technician. In cases where a laboratory is geographically isolated such that an annual visit from a technician is impractical, the certification officer may modify or waive the need for a technician.

3.3 Temperature Monitoring Device

3.3.1 Glass, dial, or electronic thermometers must be graduated in 0.5°C increments (0.2°C increments for tests which are incubated at 44.5°C) or less, except as noted for hot air ovens (3.6.1) and refrigerators (3.9.1). The fluid column in glass thermometers should not be separated. Dial thermometers that cannot be calibrated should not be used.

QC **3.3.2** The calibration of glass and electronic thermometers should be checked annually, and dial thermometers quarterly, at the temperature used, against a National Institute of Standards and Technology (NIST)-traceable reference thermometer or one that meets the requirements of NBS Monograph SP 250-23. The calibration factor and date of calibration should be indicated on the thermometer. In addition, the laboratory should record in a QC record book the following information:

- serial number of laboratory thermometer
- serial number of NIST-traceable thermometer (or other reference thermometer)
- temperature of laboratory thermometer
- temperature of NIST-traceable thermometer (or other reference thermometer)
- correction (or calibration) factor
- date of check
- analyst's initials

QC **3.3.3** If a thermometer differs by more than 1°C from the reference thermometer, it should be discarded. Reference thermometers should be recalibrated at least every five years. Reference thermometer calibration documentation should be maintained.

QC **3.3.4** Continuous recording devices that are used to monitor incubator temperature should be recalibrated at least annually. A reference thermometer that meets the specifications described in paragraph 3.3.2 should be used for calibration.

3.4 Incubator Unit

3.4.1 Incubator units must have an internal temperature monitoring device and maintain the temperature specified by the method used, usually 35°±0.5°C and 44.5°±0.2°C. For non-portable incubators, thermometers should be placed on the top and bottom shelves of the use area and immersed in liquid as directed by the manufacturer (except for electronic thermometers). If an aluminum block incubator is used, culture dishes and tubes should fit snugly. Laboratories which use the enzyme substrate tests with air-type incubators should note the product incubation details indicated in paragraph 5.3.1.5.

QC **3.4.2** Calibration-corrected temperature should be recorded for each thermometer being used at least twice per day during each day the incubator is in use, with readings separated by at least 4 hours. Documentation should include the date and time of reading, temperature, and technician's initials.

3.4.3 An incubation temperature of 44.5°±0.2°C can best be maintained with a circulating water bath equipped with a gable cover.

3.5 Autoclave

3.5.1 The autoclave should have an internal heat source, a temperature gauge with a sensor on the exhaust, a pressure gauge, and an operational safety valve. The autoclave should maintain a sterilization temperature during the sterilizing cycle and complete an entire cycle (i.e., time between starting autoclave and removing items from autoclave) within 45 minutes when a 12-15 minute sterilization period is used. The autoclave should depressurize slowly enough to ensure that media will not boil over and bubbles will not form in inverted tubes.

3.5.2 Because of safety concerns and difficulties with operational control, pressure cookers should not be used.

QC **3.5.3** The date, contents, sterilization time and temperature, total time in autoclave, and analyst's initials should be recorded each time the autoclave is used. Copies of the service contract or internal maintenance protocol and maintenance records should be kept. Maintenance should be conducted at least annually. A record of the most recent service performed should be on file, available for inspection.

QC 3.5.4 A maximum-temperature-registering thermometer, electronic temperature readout device, or continuous recording device should be used during each autoclave cycle to ensure that the proper temperature was reached, and the temperature recorded. Overcrowding should be avoided. Spore strips or spore ampules should be used monthly as bioindicators to confirm sterilization. (Since chemical indicators will respond to a wide range of times and temperatures, i.e., a longer time at a lower temperature, as well as a shorter time at a higher temperature, a positive result with the indicator does not necessarily show that sterilization has occurred.)

QC 3.5.5 Automatic timing mechanisms should be checked quarterly with a stopwatch or other accurate timepiece or time signal, and the results recorded and initialed.

3.5.6 Autoclave door seals should be clean and free of caramelized media. Also, autoclave drain screens should be cleaned frequently and debris removed.

3.6 Hot Air Oven

3.6.1 The oven should maintain a stable sterilization temperature of 170°-180°C for at least two hours. Overcrowding should be avoided. The oven thermometer should be graduated in 10°C increments or less, with the bulb placed in sand during use.

QC 3.6.2 The date, contents, sterilization time and temperature, and analyst's initials should be recorded.

QC 3.6.3 Spore strips should be used monthly to confirm sterilization. Ampules are not recommended for hot air ovens because they may explode or melt.

3.7 Colony Counter

A dark field colony counter should be used to count Heterotrophic Plate Count colonies.

3.8 Conductivity Meter

3.8.1 Meters should be suitable for checking laboratory reagent-grade water and readable in units of either micromhos/cm or microsiemens/cm.

QC 3.8.2 Calibrate the meter at least monthly, following the manufacturer's recommendations and using an appropriate certified and traceable low-level standard. If the meter cannot be calibrated with a commercial standard, the cell constant should be determined at monthly intervals, using a method in Section 2510, "Conductivity," in *Standard Methods.*

3.8.3 If an in-line unit cannot be calibrated, it should not be used to check reagent-grade water.

3.9 Refrigerator

3.9.1 Refrigerators should maintain a temperature of 1°-5°C. Calibrated thermometers should be graduated in at least 1°C increments and the thermometer bulb immersed in liquid.

QC 3.9.2 On days the refrigerator is in use, and the laboratory is staffed, the calibrated-corrected temperature should be recorded at least once per day.

3.10 Inoculating Equipment

Sterile metal or disposable plastic loops, wood applicator sticks, sterile swabs, or sterile plastic disposable pipet tips should be used. If wood applicator sticks are used, they should be sterilized by dry heat. The metal inoculating loops and/or needles should be made of nickel alloy or platinum. (When performing an oxidase test, do not use nickel alloy loops because they may interfere with the test).

3.11 Membrane Filtration Equipment (if MF procedure is used)

3.11.1 MF units must be stainless steel, glass, porcelain, or autoclavable plastic, not scratched or corroded, and must not leak.

QC **3.11.2** If graduation marks on clear glass or plastic funnels are used to measure sample volume, their accuracy should be checked with a Class B graduated cylinder or better (or other Class B glassware), and a record of this calibration check retained.

3.11.3 A 10X to 15X stereo microscope with a fluorescent light source must be used to count the target colonies (e.g., sheen colonies on M-Endo or Endo LES media).

3.11.4 Membrane filters must be approved by the manufacturer for total coliform water analysis. Approval is based on data from tests for toxicity, recovery, retention, and absence of growth-promoting substances. Filters must be gridmarked, 47 mm diameter, and 0.45 μm pore size, or alternate pore sizes if the manufacturer provides performance data equal to or better than the 0.45 μm pore size. They should also be white, and of celluose ester. Membrane filters and pads must be purchased presterilized or autoclaved for 10 minutes at 121°C before use.

QC **3.11.5** The lot number for membrane filters and the date received should be recorded. Ensure that membrane filters are not brittle or distorted, and that manufacturer's specification/certification sheet is available.

3.11.6 Forceps used should be blunt and smooth-tipped without corrugations on the inner sides of tips.

3.12 Culture Dishes (loose or tight lids)

3.12.1 Presterilized plastic or sterilizable glass culture dishes should be used. To maintain sterility of glass culture dishes, use stainless steel or aluminum canisters, or a wrap of heavy aluminum foil or char-resistant paper.

3.12.2 Loose-lid petri dishes should be incubated in a tight-fitting container, e.g., plastic vegetable crisper containing a moistened paper towel to prevent dehydration of membrane filter and medium.

3.12.3 Opened packs of disposable culture dishes should be resealed between use periods.

3.12.4 For membrane filter methods, culture dishes should be of an appropriate size to allow for the transfer of a single membrane per plate.

3.13 Pipets

3.13.1 To sterilize and maintain sterility of glass pipets, stainless steel or aluminum canisters should be used, or individual pipets should be wrapped in char-resistant paper or aluminum foil.

3.13.2 Pipets should have legible markings and should not be chipped or etched.

3.13.3 Opened packs of disposable sterile pipets should be resealed between use periods.

3.13.4 Pipets delivering volumes of 10 mL or less must be accurate to within a 2.5% tolerance.

QC **3.13.5** Calibrated micropipetters may be used if tips are sterile. Micropipetters should be calibrated annually and adjusted or replaced if the precision or accuracy is greater than 2.5%.

3.14 Glassware and Plasticware

3.14.1 Glassware should be borosilicate glass or other corrosion-resistant glass and free of chips and cracks. Markings on graduated cylinders and pipets must be legible. Plastic items should be clear and non-toxic to microorganisms.

QC **3.14.2** Graduated cylinders for measurement of sample volumes must be accurate to within a 2.5% tolerance. In lieu of graduated cylinders, precalibrated containers that have clearly marked volumes accurate to within a 2.5% tolerance may be used.

3.14.3 Culture tubes and containers containing fermentation medium should be of sufficient size to contain medium plus sample without being more than three quarters full.

3.14.4 Tube closures should be stainless steel, plastic, aluminum, or screw caps with non-toxic liners. Cotton plugs and foam plugs should not be used.

3.15 Sample Containers

3.15.1 Sample containers should be wide-mouth plastic or non-corrosive glass bottles with non-leaking ground glass stoppers or caps with non-toxic liners that should withstand repeated sterilization, or sterile plastic bags containing sodium thiosulfate. Other appropriate sample containers may be used. The capacity of sample containers should be at least 120 mL (4 oz.) to allow at least a 1-inch head space.

3.15.2 Glass stoppers must be covered with aluminum foil or char-resistant paper for sterilization.

3.15.3 Glass and plastic bottles that have not been presterilized should be sterilized by autoclaving. Glass bottles may also be sterilized by dry heat. Empty containers should be moistened with several drops of water before autoclaving to prevent an "air lock" sterilization failure.

3.15.4 If chlorinated water is to be analyzed, sufficient sodium thiosulfate ($Na_2S_2O_3$) must be added to the sample bottle before sterilization to neutralize any residual chlorine in the water sample. Dechlorination is addressed in Section 9060A of *Standard Methods*.

3.16 Ultraviolet lamp (if used)

3.16.1 A germicidal unit (254-nm) should be disconnected monthly and the lamp cleaned by wiping with a soft cloth moistened with ethanol. A longwave unit (365-366-nm), used for fluorometric tests, should also be kept clean.

QC **3.16.2** A germicidal unit should be tested quarterly with a UV light meter or agar spread plate. The lamp should be replaced if it emits less than 70% of its initial output or if an agar spread plate containing 200 to 250 microorganisms, exposed to the UV light for two minutes, does not show a count reduction of 99%. Other methods may be used to test a lamp if data demonstrate that they are as effective as the two suggested methods. (UV protective eye wear should be used when checking the operation of a 254-nm lamp.)

3.17 Spectrophotometer or colorimeter (if used)

3.17.1 Wavelengths should be in the visible range—Spectronic 20 (Thermo Spectronic), or equivalent, with cell holder for ½" diameter cuvettes (Model # 4015) or 13 mm × 100 mm cuvettes.

QC **3.17.2** A calibration standard and a method-specific blank should be analyzed every day the instrument is used, prior to sample analysis. The calibration standard should give a reading in the desired absorbance range and should be obtained from an outside source.

4. General Laboratory Practices

Although safety criteria are not covered in the laboratory certification program, laboratory personnel should be aware of general and customary safety practices for laboratories. Each laboratory is encouraged to have a safety plan available. Also, each laboratory should keep a copy, and follow the personal protection guidelines, of any material safety data sheet accompanying the receipt of a toxic material.

4.1 Sterilization Procedures

4.1.1 Autoclaving times at 121°C are listed below. Except for membrane filters and pads and carbohydrate-containing media, indicated times are minimum times and may necessitate adjustment depending upon volumes, containers, and loads. Carbohydrate-based media should not be over-sterilized.

Item	Time (min)
Membrane filters & pads	10
Carbohydrate containing media	12-15[1]
Contaminated test materials	30[2]
Membrane filter assemblies	15
Sample collection bottles	15
Individual glassware	15
Dilution water blank	15
Rinse water (0.5 - 1 L)	15-30[2]

[1] except when otherwise specified by the manufacturer
[2] time depends upon water volume per container and autoclave load

4.1.2 Autoclaved membrane filters and pads and all media should be removed immediately after completion of the sterilization cycle.

4.1.3 Membrane filter equipment must be autoclaved before the beginning of a filtration series. A filtration series ends when 30 minutes or longer elapses after a sample is filtered.

4.1.4 Ultraviolet light (254 nm) may be used to sanitize equipment (after initial autoclaving for sterilization), if all supplies are presterilized. Ultraviolet light may be used to reduce bacterial carry-over between samples during a filtration series.

4.2 Sample Containers

QC At least one sample container should be selected at random from each batch of sterile sample bottles or other containers (or lot of commercially available sample containers), and the sterility confirmed by adding approximately 25 mL of a sterile non-selective broth (e.g., tryptic soy, trypticase soy, or tryptone broth). The broth should be incubated at 35°±0.5°C, and checked after 24 and 48 hours for growth. Record results. Resterilize entire batch if growth is detected.

4.3 Reagent-Grade Water

4.3.1 Only satisfactorily tested reagent water from stills or deionization units may be used to prepare media, reagents, and dilution/rinse water for performing microbial analyses.

QC 4.3.2 The quality of the reagent water should be tested and should meet the following criteria:

Parameter	Limits	Frequency
Conductivity	>0.5 megohms resistance or <2 micromhos/cm (microsiemens/cm) at 25°C	Monthly[4]
Pb, Cd, Cr, Cu, Ni, Zn	Not greater than 0.05 mg/L per contaminant. Collectively, no greater than 0.1 mg/L	Annually
Total Chlorine Residual[1]	<0.1 mg/L	Monthly
Heterotrophic Plate Count[2]	< 500 CFU/mL[5]	Monthly

Parameter	Limits	Frequency
Bacteriological Quality of Reagent Water[3]	Ratio of growth rate 0.8 to 3.0	Annually

[1] DPD Method should be used. Not required if source water is not chlorinated.
[2] Pour Plate Method. See *Standard Methods* 9215B.
[3] See *Standard Methods* (18th or 19th eds.), Section 9020B, under *Laboratory Supplies*. This bacteriological quality test is not needed for Type II water or better, as defined in *Standard Methods* (18th and 19th eds), Section 1080C, or Medium quality water or better, as defined in *Standard Methods* (20th ed.), Section 1080C. If Type II or Medium quality water or better is not available, and a glass still is used for reagent water, a silicon test that meets the specifications of *Standard Methods*, Section 1080C (20th ed.) should also be accomplished.
[4] Monthly, if meter is in-line or has a resistivity indicator light; otherwise, with each new batch of reagent water.
[5] CFU means colony-forming units (same as colonies, but is a more precise term).

4.4 Dilution/Rinse Water

4.4.1 Stock buffer solution or peptone water should be prepared, as specified in *Standard Methods*, Section 9050C.

4.4.2 Stock buffers should be autoclaved or filter-sterilized, and containers should be labeled and dated. Stock buffers should be refrigerated. Stored stock buffers should be free from turbidity.

QC **4.4.3** Each batch (or lot, if commercially prepared) of dilution/rinse water should be checked for sterility by adding 50 mL of water to 50 mL of a double strength non-selective broth (e.g., tryptic soy, trypticase soy or tryptose broth). Incubate at 35°±0.5°C, and check for growth after 24 and 48 hours. Record results. Discard batch if growth is detected.

4.5 Glassware Washing

4.5.1 Distilled or deionized water should be used for final rinse.

4.5.2 Laboratory glassware should be washed with a detergent designed for laboratory use.

QC **4.5.3** A glassware inhibitory residue test (*Standard Methods*, Section 9020B, under *Laboratory Supplies*) should be performed before the initial use of a washing compound and whenever a different formulation of washing compound, or washing procedure, is used. Record results. This test will ensure that glassware is free of toxic residue.

QC **4.5.4** Each batch of dry glassware used for microbial analysis should be checked for pH reaction, especially if glassware is soaked in alkali or acid (*Standard Methods*, Section 9020B, under *Laboratory Supplies*). Use 0.04% bromthymol blue (or equivalent pH indicator) and observe color reaction. Clean glassware without an alkali or acid residual should have a neutral color reaction (blue-green for bromthymol blue). Record results. This test will ensure that glassware is at a neutral pH.

5. Analytical Methodology

5.1 General

5.1.1 For compliance samples, laboratories must use only the analytical methodology specified in the Total Coliform Rule (40 CFR 141.21(f)), the Surface Water Treatment Rule (SWTR) (40 CFR 141.74(a)), and the Groundwater Rule (TBD). For convenience, these regulations are reproduced in Appendix G.

5.1.2 A laboratory must be certified for all analytical methods that it uses for compliance purposes. At a minimum, the laboratory must be certified for one total coliform method and one fecal coliform or *E. coli* method. A laboratory should also be certified for a second total coliform method if one method cannot be used for some drinking waters (e.g., where the water usually produces confluent growth on a plate). In addition, for laboratories that may enumerate heterotrophic bacteria (as measured by the Heterotrophic Plate Count, HPC) for compliance with the Surface Water Treatment Rule, the laboratory must be certified either for the Pour Plate Method or the SimPlate method for heterotrophic bacteria.

5.1.3 Water samples should be shaken vigorously at least 25 times before analyzing.

QC 5.1.4 If dilution buffer is used, check the accuracy of the buffer volume in one dilution bottle in each batch or lot. For a 90-mL or 99-mL volume, the tolerance should be ±2 mL.

5.1.5 Sample volume analyzed for total coliforms in drinking water must be 100 mL. To assure accuracy and consistency within methods and between methods it is important that the laboratory obtain precise measurement of the volume of sample to be analyzed. To ensure that the required volume of 100 mL is analyzed, no matter which of the approved methods the laboratory will be employing for analysis, good laboratory practice dictates that a sterile, calibrated measuring vessel be used for measurement of the sample volume. It is inappropriate for a portion of the sample to be poured to waste in order to meet the required sample volume, as this practice could easily result in laboratory error which could then require the sample to be invalidated.

5.1.6 Media (or defined substrate)

5.1.6.1 The use of dehydrated or prepared media manufactured commercially is strongly recommended due to concern about quality control. Dehydrated media should be stored in a cool, dry location, and discarded by manufacturer's expiration date. Caked or discolored dehydrated media should be discarded.

QC 5.1.6.2 For media prepared in the laboratory, the date of preparation, type of medium, lot number, sterilization time and temperature, final pH (after sterilization), and the technician's initials should be recorded.

QC 5.1.6.3 For media prepared commercially, the date received, type of medium, lot number, and (if identified by the manufacturer or method) pH verification for each lot should be recorded. Media should be discarded by manufacturer's expiration date.

QC 5.1.6.4 Each new lot of dehydrated or prepared commercial medium and each batch of laboratory-prepared medium should be checked before use for sterility and with positive and negative culture controls. Those laboratories using commercially prepared media with manufacturer shelf-lives of greater than 90 days should run positive and negative controls each quarter, in addition to running these controls and sterility checks on each new lot of media. Laboratories are encouraged to perform positive and negative control tests on a more frequent basis. Control organisms (total coliforms, fecal coliforms, and/or *E. coli*, as appropriate) can be stock cultures (periodically checked for purity) or commercially available disks impregnated with the organism. Results should be recorded. The following Table identifies a few positive and negative culture controls that laboratories might consider, although other culture controls are also acceptable.

Control Cultures for Microbiological Tests

Group	Positive Culture Control[9]	Negative Culture Control[9]
Total coliforms	*Escherichia coli* *Enterobacter aerogenes*	*Staphylococcus aureus*[1] *Proteus vulgaris*[2] *Pseudomonas aeruginosa*[1]
Fecal coliforms	*Escherichia coli* *Klebsiella pneumoniae* (thermotolerant)	*Enterobacter aerogenes*[3]
E. coli	*Escherichia coli* (MUG-positive strain)	*Enterobacter aerogenes* *Klebsiella pneumoniae*[4] (thermotolerant)
Enterococci[5]	*Enterococcus faecalis* *Enterococcus faecium*	*Staphylococcus aureus*[6] *E. coli*[7] *Serratia marcesens*[8]

[1] *S. aureus, P. aeruginosa* - not lactose fermenter

[2] *P. vulgaris* - not lactose fermenter; uses hydrolyzed lactose, indicating "overcooked" medium

[3] *E. aerogenes* - ferments lactose, but is not typically thermotolerant

[4] *K. pneumoniae* - ferments lactose, but does not hydrolyze MUG

[5] Do not use closely related strains from genus *Streptococcus* as a positive control

[6] *S. aureus* - sensitive to nalidixic acid in medium

[7] *E. coli* - sensitive to sodium azide in medium

[8] *S. marcescens* - will not hydrolyze fluorogenic compound in medium

[9] Examples of appropriate ATCC strains include the following:

Enterococcus faecalis ATCC 11700
Enterobacter aerogenes ATCC 13048
Klebsiella pneumoniae (thermotolerant) ATCC 13883
Pseudomonas aeruginosa ATCC 27853
Staphylococcus aureus ATCC 6538
Enterococcus faecium ATCC 6057
Escherichia coli ATCC 8739 or 25922
Proteus vulgaris ATCC 13315
Serratia marcesens ATCC 14756

5.1.6.5 If prepared medium is stored after sterilization, it should be maintained in the dark, avoiding moisture loss, per the following Table. Prepared plates may be stored in sealed plastic bags or containers. For either broth or agar media, each bag or container should include the date prepared or an expiration date. If the medium is stored in a refrigerator, it should be warmed to room temperature before use; tubes or plates that show growth and/or bubbles should be discarded. Liquid media should be discarded if evaporation exceeds 10% of the original volume.

Maximum Holding Times and Temperatures for Prepared Media

Container	Max storage temp.	Max. storage time
Poured agar plates	1-5°C	2 weeks
Broth in tubes, bottles, or flasks with loose-fitting closures	1-30°C	2 weeks
Broth in tightly closed screw-cap tubes, bottles, or flasks	1-30°C	3 months

QC **5.1.7** Laboratories are encouraged to perform parallel testing between a newly approved test and another EPA-approved procedure for enumerating total coliforms for at least several months and/or over several seasons to assess the effectiveness of the new test for the wide variety of water types submitted for analysis. During this testing, spiking the samples occasionally with sewage or a pure culture may be necessary to ensure that some of the tests are positive.

5.1.8 A list of approved analytical methods (or proposed methods, where noted), applicable regulations, and section identifiers for each method is provided in the Table below.

Approved Methods	Part	Media	Method Citation[1]	TCR[2] (Detect)	SWTR[2] (Count)	GWR[2] (Detect)
Total Coliforms						
Fermentation broth method	5.2.2	LTB⇒BGLB Broth	SM 9221B,C	X	X	
	5.2.3	P-A Broth ⇒ BGLB Broth	SM 9221D	X		
Enzyme substrate method	5.3.2	Colilert®, Colilert-18®	SM 9223	X	X	
	5.3.2	Colisure®	SM 9223	X		
	5.3.2	Readycult® or Fluorocult LMX®		X		
	5.3.2	E*Colite®		X		
	5.3.2	Colitag®		X		
Membrane filter method	5.4.2	M-Endo or LES-Endo ⇒ LTB, BGLB Broth	SM 9222B,C	X	X	
	5.4.2	MI Medium	SM 9222	X	X	
	5.4.2	m-ColiBlue 24®		X		
	5.4.2	Chromocult®		X		
	5.4.2	Coliscan®		X	X	
Fecal Coliforms						
Fermentation broth method	5.2.4	LTB or P/A broth ⇒ EC broth	(SM 9221B,D) SM 9221E	X	X	
	5.2.4	A-1 broth	SM 9221E		X	
Membrane filter method	5.2.4	M-Endo medium ⇒ EC broth	(SM 9222B) SM 9221E	X	X	
	5.4.2	mFC	SM 9222D		X	

Approved Methods	Part	Media	Method Citation[1]	TCR[2] (Detect)	SWTR[2] (Count)	GWR[2] (Detect)
Escherichia coli						
Enzyme substrate method	5.3.2	Colilert® or Colilert-18®	SM 9223	X		X
	5.3.2	Colisure®	SM 9223	X		X
	5.3.2	E*Colite®		X		X
	5.3.2	Readycult® or Fluorocult LMX®		X		
	5.3.3	LTB, P/A broth, M-Endo ⇒ EC-MUG	(SM 9221B,D; SM 9222B) SM 9221F	X		X
	5.3.2	Colitag®		X		
Membrane filter method	5.4.2	MI Medium	SM 9222	X		X
	5.4.2	m-ColiBlue24®		X		X
	5.4.2	Chromocult®		X		
	5.4.2	Coliscan®		X		
	5.4.3	M-Endo or LES Endo ⇒ NA-MUG	(SM 9222B) → SM 9222G	X		X
Enterococci[3]						
Enzyme substrate method	5.3.4	Enterolert	ASTM D6503-99			X
Fermentation broth method	5.2.5	Azide Dextrose ⇒ BEA/BHI	SM 9230B			X
Membrane filter method	5.4.4	mE ⇒EIA m-Enterococcus	SM 9230C			X
	5.4.4	mEI	EPA 1600			X
Heterotrophic Bacteria						
Pour plate method	5.5	Plate count agar	SM 9215B		X	
Multiple enzyme substrate	5.5	SimPlate®			X	
Pour plate, spread plate, or membrane filter methods	5.5	R2A		X[4]		

Approved Methods	Part	Media	Method Citation[1]	TCR[2] (Detect)	SWTR[2] (Count)	GWR[2] (Detect)
Male-Specific and Somatic Coliphage[3]						
Agar plate method	5.6.2	Two-Step Enrichment	EPA 1601			X
	5.6.3	Single Agar Layer	EPA 1602			X

[1] SM = *Standard Methods for the Examination of Water and Wastewater*, 18th, 19th or 20th edition.
[2] TCR=Total Coliform Rule (40 CFR 141.21 (f)), SWTR=Surface Water Treatment Rule (40 CFR 141.74 (a)). For convenience, analytical methods approved for the TCR and SWTR are reproduced in Appendix G.
[3] GWR = Based on proposed Groundwater Rule (65 FR 30194, dated 5/10/2000). Until the GWR is promulgated, laboratories will not be certified for enterococci or coliphage methods.
[4] For possible use if system operates under a variance to the TCR.

5.2 Fermentation broth methods

5.2.1 General

5.2.1.1 The water level of the water bath should be above the upper level of the medium in the culture tubes.

5.2.1.2 A Dri-bath incubator is acceptable if the specified temperature requirement can be maintained in all tube locations used.

5.2.2 Multiple Tube Fermentation Technique (for detecting total coliforms in drinking water and enumerating total coliforms in source water)

5.2.2.1 For drinking water samples: Various testing configurations can be used (CFR141.21(f)(3), see Appendix G), as long as a total sample volume of 100 mL is examined for each test.

5.2.2.2 For source water samples: Laboratories must use at least 3 series of 5 tubes each with appropriate sample dilutions of source water (e.g., 0.1 mL, 0.01 mL, 0.001 mL).

5.2.2.3 Media

5.2.2.3.1 Lauryl tryptose broth (LTB) (also known as lauryl sulfate broth) must be used in the presumptive test and 2% brilliant green lactose bile broth (BGLBB) in the confirmed test. Lactose broth (LB) may be used in lieu of LTB (40 CFR 141.21(f)(3)) if the laboratory conducts at least 25 parallel tests between this medium and LTB using the waters normally tested and this comparison demonstrates that the false-positive rate and false-negative rate for total coliforms, using LB, is less than 10%. This comparison should be documented and the records retained. The final pH must be 6.8±0.2 for LTB, and 7.2±0.2 for 2% BGLBB.

5.2.2.3.2 The test medium concentration must be adjusted to compensate for the sample volume so that the resulting medium after sample addition is single strength. Optionally, if a single 100-mL sample volume is used, the inverted vial should be replaced with an acid indicator (bromcresol purple) to prevent problems associated with gas bubbles in large inverted tubes. The media must be autoclaved at 121°C for 12-15 minutes.

5.2.2.3.3 Sterile medium in tubes must be examined to ensure that the inverted vials, if used, are free of air bubbles and are at least one-half to two-thirds covered after the water sample is added.

5.2.2.4 After the medium is inoculated, it must be incubated at 35°±0.5°C for 24±2 hours. If no gas or acid is detected, it must be incubated for another 24 hours (total incubation time 48±3 hours).

5.2.2.5 Each 24- and 48-hour tube that contains growth, acid, or gas must be confirmed using 2% BGLBB. A completed test is not required.

5.2.2.6 For drinking water samples: Test each total coliform-positive sample for the presence of either fecal coliforms or *E. coli.*

5.2.2.7 Invalidation of total coliform-negative samples

5.2.2.7.1 For drinking water samples: All samples that produce a turbid culture (i.e., heavy growth) in the absence of gas/acid production, in LTB or LB, must be invalidated. The laboratory must collect, or request that the system collect, another sample within 24 hours from the same location as the original invalidated sample. (Before invalidation, the laboratory may perform a confirmed test and/or a fecal coliform/*E. coli* test on the total coliform-negative culture to check for coliform suppression. If the confirmed test is total coliform-positive or if fecal coliforms/*E. coli* are detected, the sample must be reported as such. A fecal coliform/*E. coli*- positive result is considered a total coliform-positive, fecal coliform/*E. coli*-positive sample, even if the presumptive or confirmed total coliform test is negative. If the follow-up test(s) is negative, the sample must be invalidated because high levels of non-coliform bacteria in the presumptive tubes may have injured, killed, or suppressed the growth of any coliforms in the sample.)

5.2.2.7.2 For source water samples: All samples that produce a turbid culture (i.e., heavy growth) in the absence of gas/acid production, in LTB or LB, should be invalidated. The laboratory should collect, or request that the system collect, another sample from the same location as the original invalidated sample. (Before invalidation, the laboratory may perform a confirmed test on the total coliform-negative culture. If the confirmed test is total coliform-positive, the MPN should be reported. If the test is total coliform-negative, the sample should be invalidated.)

5.2.3 Presence-Absence (P-A) Coliform Test (for detecting total coliforms in drinking water)

5.2.3.1 Medium

5.2.3.1.1 Six-times formulation strength may be used. If the 6-times formulation is used, it must be filter-sterilized rather than autoclaved.

5.2.3.1.2 The medium must be autoclaved for 12 minutes at 121°C. Total time in the autoclave should be less than 30 minutes. Space should be allowed between bottles. The final pH must be 6.8±0.2.

5.2.3.1.3 If prepared medium is stored, it should be maintained in a culture bottle at 1°-30°C in the dark for no longer than three months. If evaporation exceeds 10% of original volume, the medium should be discarded.

5.2.3.2 A 100-mL sample must be inoculated into a P-A culture bottle.

5.2.3.3 Medium must be incubated at 35°±0.5°C and observed for a yellow color (acid) after 24 and 48 hours.

5.2.3.4 Yellow cultures must be confirmed in BGLBB and a fecal coliform/*E. coli* test conducted.

5.2.3.5 All samples which produce a non-yellow turbid culture in P-A medium must be invalidated. The laboratory must collect, or request that the system collect, another sample from the same location as the original invalidated sample. (Before invalidation, the laboratory may perform a confirmed test on the total coliform negative culture and/or a fecal coliform/*E. coli* test. If the confirmed test is total coliform-positive, the sample must be reported as such. If the confirmed test is negative, the sample must be invalidated. A fecal coliform/*E. coli* positive result is considered a total coliform-positive, fecal coliform/*E. coli* positive sample, even if the presumptive and/or confirmed total coliform test is negative.)

5.2.4 Fecal Coliform Test (using EC Medium for fecal coliforms in drinking water or source water, or A-1 Medium for fecal coliforms in source water only)

5.2.4.1 EC Medium

5.2.4.1.1 Use EC medium to test a total coliform-positive culture for fecal coliforms under the Total Coliform Rule. The laboratory must transfer each total coliform-positive culture from a presumptive tube/bottle, or each presumptive total coliform-positive colony unless a cotton swab is used, to at least one tube containing EC Medium with an inverted vial, as specified by §141.21(f)(5)(See Appendix G).

5.2.4.1.2 EC Medium may be used to enumerate fecal coliforms in source water, in accordance with the Surface Water Treatment Rule. Initially, conduct a MTF test (presumptive phase). Three sample volumes of source water (e.g., 10, 1 and 0.1 mL), 5 or 10 tubes/sample volume, should be used. A culture from each total coliform-positive tube should be transferred to a tube containing EC Medium with an inverted vial.

5.2.4.1.3 Autoclave EC Medium for 12-15 minutes at 121°C. The final pH should be 6.9±0.2.

5.2.4.1.4 Inverted vials should be examined to ensure that they are free of air bubbles. The inverted vial must be at least one-half to two-thirds covered after the sample is added.

5.2.4.1.5 EC Medium must be incubated at 44.5°±0.2°C for 24±2 hours.

5.2.4.1.6 Any amount of gas detected in the inverted vial of a tube that has turbid growth must be considered a fecal coliform-positive test, regardless of the result of any subsequent test on that culture.

5.2.4.2 A-1 Medium

5.2.4.2.1 A-1 medium may be used as an alternative to EC Medium to enumerate fecal coliforms in source water, in accordance with the Surface Water Treatment Rule. A-1 Medium must not be used for drinking water samples. Three sample volumes of source water (e.g., 10, 1 and 0.1 mL), 5 or 10 tubes/sample volume, should be used. Unlike EC Medium, A-1 Medium may be used for the direct isolation of fecal coliforms from water.

5.2.4.2.2 A-1 Medium must be sterilized by autoclaving at 121°C for 10 minutes. The final pH must be 6.9±0.1.

5.2.4.2.3 Inverted vials should be examined to ensure that they are free of air bubbles.

5.2.4.2.4 A-1 Medium must be incubated at 35°±0.5°C for three hours, then at 44.5°±0.2°C for 21±2 hours.

5.2.4.2.5 Loose-cap tubes should be stored in dark at room temperature not more than two weeks. A-1 Medium must not be held more than three months in tightly closed screw-cap tubes in the dark at 4°C.

5.2.4.3 Any amount of gas detected in the inverted vial of a tube that has turbid growth must be considered a fecal coliform-positive test, regardless of the result of any subsequent test on that culture.

5.2.5 Azide dextrose medium (for detecting fecal streptococci in ground water)

5.2.5.1 For testing 100-mL samples, prepare triple strength (3X) formulation in a culture bottle and autoclave at 121°C for 15 minutes. Final pH should be 7.2±0.2.

5.2.5.2 Add a 100-mL water sample to the sterilized medium, and incubate at 35°±0.5°C.

5.2.5.3 Check culture for turbidity after 24±2 hours. If turbidity is not observed, reincubate and check again after a total incubation period of 48±3 hours.

5.2.5.4 A turbid culture may be confirmed as fecal streptococci by streaking a portion of the broth onto bile esculin agar (BEA) or bile esculin azide agar (BEAA). (The confirmation medium in *Standard Methods*, PSE Medium, is no longer commercially available.)

5.2.5.5 Before streaking, BEA and BEAA must be sterilized by autoclaving at 121°C for 15 minutes. Final pH should be 6.6±0.2 for BEA and 7.1±0.2 for BEAA.

5.2.5.6 After streaking, BEA and BEAA plates must be incubated at 35°±0.5°C for 48 hours.

5.2.5.7 Brownish-black colonies on BEA or BEAA with brown halos confirm the presence of fecal streptococci. If required, an enterococci test can be performed on one or more fecal streptococci colonies by transferring them to brain heart infusion broth supplemented with 6.5% NaCl, and incubating the culture at 35°±0.5°C for 48 hours. Growth indicates the presence of enterococci.

5.3 Enzyme (Chromogenic/fluorogenic) substrate tests

5.3.1 General

5.3.1.1 For detecting total coliforms and *E. coli* in drinking water samples, a laboratory may use the MMO-MUG test (Colilert), Colisure test, E*Colite test, Readycult Coliforms 100 Presence/Absence Test (or Fluorocult LMX Broth test), or Colitag test. These tests may be available in various configurations. For enumerating total coliforms in source waters, a laboratory may use the Colilert test. If a laboratory uses a fermentation method to detect total coliforms in drinking water, and the sample is total coliform-positive, the laboratory may transfer the positive culture to the EC+MUG test to detect *E. coli*, but not to any other enzyme substrate test medium in this section.

5.3.1.2 Media

5.3.1.2.1 Media must not be prepared from basic ingredients, but rather purchased from a commercially available source.

5.3.1.2.2. The media must be protected from light.

5.3.1.2.3 Some lots of enzyme substrate media have been known to fluoresce. Therefore, each lot of medium should be checked before use with a 365-366-nm ultraviolet light with a 6-watt bulb. For checking Colilert, Colilert-18, Colisure, Readycult/Fluorocult LMX, and Colitag media, a packet of medium should be dissolved in sterile water in a non-fluorescing vessel. If the medium exhibits faint fluorescence, the laboratory should use another lot that does not fluoresce.

5.3.1.2.4 If the samples plus a medium exhibit an inappropriate color change before incubation, it should be discarded and another lot of medium used. The laboratory should notify the medium vendor and request another water sample from the water system. Before incubation, Colilert, Colilert-18, and Colitag should appear colorless to a slight tinge of color, while Colisure and E*Colite are yellow and Readycult/Fluorocult is slightly yellow.

5.3.1.3 Glass and plastic bottles and test tubes should be tested before use with a 365-366-nm ultraviolet light source with a 6-watt bulb to ensure they do not fluoresce. If they fluoresce, use another lot of containers that do not fluoresce.

5.3.1.4 If a Whirl-Pak® bag is used to incubate the Colilert or Colitag medium or any other medium which changes to a yellow color to indicate a positive result, use a type that has a barrier (e.g., B01417) to prevent gaseous emissions to other Whirl-Pak® bags during incubation.

QC **5.3.1.5** Incubators, especially small, low wattage air-type incubators, may not bring a cold 100-mL water sample(s) to the specified incubation temperature for several hours. The problem may cause false-negative results with the enzyme substrate tests and possibly other tests as well. Therefore, laboratories with air-type incubators should observe the following instructions for chromogenic/fluorogenic substrate tests:

Test	Pre-incubation sample instructions[1,2]
Colilert (Presence/Absence)	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35°C[1]
Colilert Quanti-Tray	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35°C
Colilert-18 (Presence/Absence)	Prewarm sample in 35°C water bath for 20 minutes or 44.5°C for 7-10 minutes
Colilert-18 Quanti-Tray	Allow sample to equilibrate to room temperature (20-30°C) before beginning 18-hour incubation time
Colisure	Allow sample to equilibrate to room temperature (20-30°C) before beginning 24-hour incubation time
Readycult Coliforms 100 Presence/Absence Test and Fluorocult LMX Broth	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35°±0.5°C
Colitag	Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35°±0.5°C

[1] If the laboratory plans to put a large load into a small incubator, samples should be brought to room temperature before incubation.
[2] Information based on manufacturer's instructions.

5.3.1.6 If a water bath is used, the water level should be above the upper level of the medium.

5.3.1.7 For *E. coli* testing, the laboratory must place all total coliform-positive samples under an ultraviolet lamp (365-366 nm, 6-watt) in a darkened area. If *E. coli* is present, the medium will emit a blue fluorescence.

5.3.1.8 The enzyme substrate tests should not be used to confirm a presumptive total coliform-positive culture in fermentation broth (e.g., LTB, LB, P-A coliform test) or on a membrane filter. The high densities of non-coliforms or turbidity in the inoculum may either suppress coliforms or overload the enzyme substrate test suppressant reagent system and cause false-positive results.

5.3.1.9 Any sample that produces an atypical color change (e.g., greenish-black or black) in the absence of a yellow color should be invalidated. The laboratory must collect, or request that the system collect, another sample from the same location as the original invalidated sample. The laboratory should use another method to test the second sample. According to the manufacturer of Colilert, water with high iron or manganese levels in the presence of hydrogen sulfide may cause a greenish-black or black color. This greenish-black color does not occur when using Readycult, Colisure, or Colitag, according to their manufacturers.

5.3.1.10 Any reference comparator provided by the manufacturer should be discarded by the manufacturer's expiration date.

5.3.2 Criteria for specific media

5.3.2.1 For the Colilert test, samples must be incubated at 35°±0.5°C for 24 hours. A yellow color in the medium equal to or greater than the reference comparator indicates that the sample is total coliform-positive. If the sample is yellow, but lighter than the comparator, it must be incubated for another four hours (do not incubate more than 28 hours total). If the color is still lighter than the reference comparator at 28 hours, the sample should be reported as negative. A coliform-positive sample that fluoresces under a UV light indicates the presence of *E. coli*. Laboratories that use the Colilert-18 test must incubate samples for 18 hours (up to 22 hours if sample after 18 hours is yellow, but is lighter than the comparator).

5.3.2.1.1 For enumerating total coliforms in source water with the Colilert test, a 5- or 10-tube configuration, Quanti-Tray, or Quanti-Tray 2000 may be used for each sample dilution tested. Dilution water (if used) may be sterile deionized or sterile distilled water, but not buffered water.

QC 5.3.2.1.2 If the Quanti-Tray or Quanti-Tray 2000 test is used, the sealer should be checked monthly by adding a dye (e.g., bromcresol purple) to the water. If dye is observed outside the wells, either perform maintenance or use another sealer.

5.3.2.2 For the Colisure test, samples must be incubated at 35°±0.5°C for 24 hours. If an examination of the results at 24 hours is not convenient, then results may be examined at any time up to 48 hours. If the medium changes from a yellow color to a red/magenta color, the sample is total coliform-positive. A coliform-positive sample that fluoresces under a UV light indicates the presence of *E. coli*.

5.3.2.3 For the E*Colite test, samples must be incubated at 35°±0.5°C for 28 hours. If total coliforms are present, the medium changes from a yellow color to a blue or blue-green color, or a blue color in the corners of the bag. If *E. coli* is present, medium will fluoresce under a UV light. If no fluorescence is observed, re-incubate for an additional 20 hours (for a total incubation time of 48 hours) and again check for fluorescence. If medium becomes red in color, assume that a faulty seal has allowed the bactericide (in the third compartment of the bag) to leak into the compartment containing the medium. In this case, discard the sample, and request another sample.

5.3.2.4 For the Readycult Coliforms 100 Presence-Absence test, the contents of a snap pack should be added to a 100-mL water sample, followed by incubation at 35°±05°C for 24±1 hours. If coliforms are present, the medium changes color from a slightly yellow color to blue-green. In addition, if *E. coli* is present, the medium will emit a bright light-blue fluorescence when subjected to a long wave (365-366 nm) ultraviolet (UV) light. If confirmation of *E. coli* is desired, Kovac's indole reagent should be added to the broth; the immediate formation of a red ring confirms the presence of *E. coli*.

5.3.2.5 Fluorocult LMX broth is identical to Readycult, except that it is a dehydrated culture medium in granulated form packed primarily in a 500 g plastic bottle. For testing a 100-mL water sample, suspend 34 g of Fluorocult LMX in 1L purified water and boil to dissolve completely. Transfer 100-mL aliquots to 250-mL bottles and autoclave for 15 min at 121°C. Cool to room temperature, add the 100-mL water sample, and incubate. Do not add *E. coli*/Coliform Supplement to the medium.

5.3.2.6 For the Colitag test, samples must be incubated at 35°±0.5°C for 24±2 hours. During incubation, trimethylamine-N-oxide in the Colitag medium causes the pH of the medium to increase from 6.2 to 6.8-7.2. A yellow color in the medium indicates the presence of total coliforms. A coliform-positive sample that fluoresces under a UV light indicates the presence of *E. coli*.

5.3.3 EC Medium + MUG Test (for detection of *E. coli*)

5.3.3.1 If EC medium + MUG is used, a total coliform-positive culture must be transferred from a presumptive tube/bottle or colony to EC medium + MUG, as specified by §141.21(f)(5)(See Appendix G).

5.3.3.2 MUG may be added to EC Medium before autoclaving. EC Medium+MUG is also available commercially. The final MUG concentration must be 50 µg/mL. The final pH should be 6.9±0.2.

5.3.3.3 The inverted vial may be omitted, because gas production is not relevant to the *E. coli* test.

5.3.3.4 The medium must be incubated at 44.5°±0.2°C for 24±2 hours, and tested for fluorescence.

5.3.4 Enterolert test (for detection of enterococci in ground water)

5.3.4.1 Medium should be stored in the dark at 4-30°C until use.

5.3.4.2 Add Enterolert reagent to 100-mL water sample, and incubate at 41°± 0.5°C for 24-28 hours. Fluorescence under a UV lamp indicates the presence of enterococci.

5.3.4.3 The development of fluorescence after 28 hours is not a valid test for enterococci.

5.4 Membrane Filter (MF) methods

5.4.1 General

5.4.1.1 For source water samples (SWTR): To optimize counting, appropriate sample dilutions must be used to yield 20 to 80 total coliform colonies or 20-60 fecal coliform colonies for at least one dilution or volume.

QC **5.4.1.2** At least one membrane filter and filtration unit sterility check should be conducted at the beginning and the end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter and testing for growth. If the control indicates contamination, all data from affected samples must be rejected and an immediate resampling should be requested. A filtration series ends when 30 minutes or more elapse between sample filtrations.

5.4.1.3 Each filtration funnel must be rinsed after each sample filtration with two or three 20-30 mL portions of sterile rinse water to ensure that entire sample is rinsed off the funnel before the filter is removed. After the filter is removed, the funnel may be rinsed again with two or three 20-30 mL portions of sterile rinse water or exposed to UV light with a 254-nm wavelength for at least two minutes to prevent carry-over between samples, especially for surface water samples..

5.4.1.4 Absorbent pads must be saturated with a liquid medium (at least 2 mL of broth) and excess medium removed by "decanting" the plate.

5.4.2 MF method for detecting total coliforms and *E. coli* in drinking water, enumerating total coliforms or fecal coliforms in source water, and detecting *E. coli* in ground water

5.4.2.1 Media for total coliforms, fecal coliforms, and *E. coli*

5.4.2.1.1 M-Endo Medium agar or broth (also known as M-Endo broth MF and M-Coliform Broth) or LES Endo agar (also known as M-Endo Agar LES) for detecting total coliforms in drinking water or enumerating total coliforms in source water. Medium may be used in the single step or enrichment techniques. Ensure that ethanol used in the rehydration procedure is not denatured. Medium should be prepared in a sterile flask and brought just to the boiling point with a boiling water bath or, if constantly attended, a hot plate with a stir bar. The medium must not be boiled. Final pH should be 7.2±0.2 for M-Endo Agar LES and 7.2±0.1 for M-Endo medium.

5.4.2.1.2 m-ColiBlue24 medium for detecting total coliforms and *E. coli* in drinking water. Ampules

of broth should be inverted 2-3 times to mix contents before breaking. Then contents should be poured evenly over absorbent pad. Unopened refrigerated ampules may be stored in the dark until the expiration date, but should be discarded earlier if growth is observed. The final pH of medium should be 7.0±0.2.

5.4.2.1.3 MI Medium (with or without agar) for detecting total coliforms and E. coli in drinking water or enumerating total coliforms in source water. Do not autoclave commercially made, presterilized bottled MI agar or broth. Melt bottled agar in a boiling water bath (or by other processes recommended by the manufacturer). As soon as complete melting has occurred, cool slightly and pour immediately into sterile plates. Care should be taken to prevent overheating the agar, as excessive heat destroys the effectiveness of the antibiotic, cefsulodin. If dehydrated culture medium is used, it should be prepared and autoclaved according to the manufacturer's instructions. Cool the agar, add freshly prepared, filter-sterilized cefsulodin, and pour immediately into sterile plates. The final pH of MI agar should be 6.95±0.20; the final pH of MI broth should be 7.05±0.20. The preparation and use of MI agar and MI broth is described in the article, "New medium for the simultaneous detection of total coliform and *Escherichia coli* in water" by Brenner, K.P., *et al.*, 1993, *Applied and Environmental Microbiology* 59:3534-3544. EPA Method 1604, which can be found online at www.epa.gov/microbes, is identical.

5.4.2.1.4 Chromocult® Coliform Agar for detecting total coliforms and *E. coli* in drinking water. Do not autoclave or overheat. The final pH should be 6.8±0.2. If a heavy background of heterotrophic bacteria is expected (especially *Pseudomonas* and *Aeromonas* spp.), add cefsulodin solution to the cooled (45°-50°C) medium (dissolve 10 mg cefsulodin in 2 mL deionized or distilled water, and add solution to 1L of medium). Check with the manufacturer, EMD Chemicals, Inc., at www.emdchemicals.com, or call (800) 222-0342 for additional information on the performance of this test with various filter types.

5.4.2.1.5 Coliscan® for detecting total coliforms and *E. coli* in drinking water or enumerating total coliforms in source water. Coliscan is available as a dry powder agar mix or as a presterilized bottled agar. For reconstitution and antibiotic addition, follow the protocol of the manufacture (Micrology Laboratories, LLC). Do not overheat the antibiotic, cefsulodin. The final pH of Coliscan agar should be 7.00±0.20.

5.4.2.1.6 m-FC broth (with or without agar) for enumerating fecal coliforms in source water. Do not autoclave. Bring medium just to the boiling point. The final pH should be 7.4±0.2.

5.4.2.1.7 When stored, prepared medium should be refrigerated. Petri dishes containing medium should be stored in a plastic bag or tightly closed container, and used within two weeks. Before use, refrigerated sterilized medium should be brought to room temperature. Plates with laboratory prepared broth medium must be discarded after 96 hours, poured MF agar plates discarded after two weeks, and ampuled M-Endo broth and other prepared media discarded in accordance with the manufacturer's expiration date. Broth, plates, or ampules should be discarded earlier if growth or (for M-Endo agar) surface sheen is observed. Record date and time prepared.

5.4.2.2 Incubation conditions and colony color of inoculated medium

Medium	Incubation	Total coliforms[1]	*E. coli*
M-Endo medium or M-Endo agar LES	35°±0.5°C for 22-24 hrs	Metallic (golden) sheen colonies (presumptive)	N/A
m-ColiBlue24	35°±0.5°C for 24 hrs	Red colonies	Blue to purple colonies
MI	35°±0.5°C for 24±2 hrs	Fluorescent colonies under UV light	Blue colonies under normal light

Medium	Incubation	Total coliforms[1]	*E. coli*
Chromocult	36°±1°C for 24±1 hrs	Salmon to red colonies	Dark-blue to violet colonies[2]
Coliscan	32°-37°C for 24-28 hrs	Pink-magenta colonies	Purple-blue colonies
m-FC	44.5°±0.2°C for 24±2 hrs	N/A	Blue colonies (fecal coliforms)

[1] Without the presence of *E. coli*. If an *E. coli* colony is present, as indicated by the last column, it should be counted as a total coliform-positive colony.

[2] If confirmation of *E. coli* is desired, add one drop of Kovac's reagent to each dark-blue to violet colony; the formation of a cherry-red color within seconds confirms the presence of *E. coli*.

5.4.2.3 Invalidation of a total coliform-negative drinking water sample: All samples resulting in confluent or TNTC (too numerous to count) growth must be invalidated unless total coliforms are detected. If no total coliforms are detected, record as "confluent growth" or "TNTC" and request an additional sample from the same sampling site. Confluent growth is defined as a continuous bacterial growth covering the entire membrane filter without evidence of total coliform-type colonies. TNTC is defined as greater than 200 colonies on the membrane filter in the absence of detectable coliforms. Laboratories must not invalidate samples when the membrane filter contains at least one coliform-type colony (i.e., sheen colony for M-Endo medium, red or blue colony for m-ColiBlue 24 agar, fluorescent or blue colony for MI agar, salmon to red or dark-blue to violet colonies for Chromocult Coliform agar, pink/magenta or blue/purple colony for Coliscan). (Before invalidation, the laboratory may perform a verification test on the total coliform-negative culture, i.e., on confluent or TNTC growth, and a fecal coliform/*E. coli* test. If the verification test is total coliform-positive, the sample must be reported as total coliform-positive. If the test is total coliform-negative, the sample must be invalidated. A fecal coliform/*E. coli*-positive result is considered a total coliform-positive, fecal coliform/*E. coli*-positive sample, even if the initial and/or verification total coliform test is negative.)

5.4.2.4 Invalidation of source water samples (SWTR): Laboratories must invalidate any sample which results in confluent growth or TNTC, even when total coliform or fecal coliform colonies are present, because coliform density must be determined.

5.4.2.5 For drinking water samples (to verify colonies on Endo-type medium): At least five typical sheen colonies and five nontypical colonies must be verified using either single strength lactose broth (LB) or lauryl tryptose broth (LTB) and then single strength 2% brilliant green lactose bile broth (BGLBB). Alternatively, sheen colonies may be verified using a cytochrome oxidase and ß-galactosidase procedure. Individual colonies can be transferred with a sterile needle or loop, or applicator stick. If no sheen colonies are observed, verify up to five red questionable sheen colonies and/or red non-sheen colonies representing different morphological types. Alternatively, wipe the entire surface of the membrane filter with a sterile cotton swab, and inoculate the verification media (LTB, then BGLBB).

5.4.2.6 For drinking water samples: Total coliform-positive colonies must be tested for *E. coli* or fecal coliforms. The membrane filter tests approved by EPA to date do not require additional media for such a test, except for those using Endo-type medium (M-Endo medium or M-Endo agar LES). EPA has approved several options for testing a total coliform-positive colony on Endo-type medium for *E. coli* or fecal coliforms. When EC Medium (for fecal coliforms) or EC Medium + MUG (for *E. coli*) is used, the colonies must be transferred by employing one of the options specified by the Total Coliform Rule at 40 CFR 141.21(f)(5)(See Appendix G). For the swab technique, a single swab can be used to inoculate a presumptive total coliform-positive culture into up to three different media (e.g., EC or EC-MUG Medium, LTB, and BGLBB, in that order). If Nutrient Agar + MUG is used, refer to paragraph 5.4.3.

5.4.2.7 For source water samples: Initial total coliform counts must be adjusted based upon verified data, as in *Standard Methods*, Section 9222B(5).

QC **5.4.2.8** For source water samples (SWTR): If two or more analysts are available, each analyst should count total coliforms or fecal coliform colonies on the same membrane monthly. Colony counts should agree within 10%.

5.4.3 Nutrient Agar + MUG Test (for detection of *E. coli* in drinking water or ground water)

5.4.3.1 Medium must be autoclaved at 121°C for 15 minutes. MUG may be added to Nutrient Agar before autoclaving. Nutrient Agar + MUG is also available commercially. The final MUG concentration must be 100 µg/mL. The final pH should be 6.8±0.2.

QC **5.4.3.2** Positive and negative controls should be tested as stated in paragraph 5.1.6.4. Filter or spot-inoculate control cultures onto a membrane filter on M-Endo agar LES or M-Endo broth or agar, and incubate at 35°±0.5° C for 24 hours. Then transfer the filter to Nutrient Agar + MUG and incubate at 35°C for another four hours. The results should be read and recorded.

5.4.3.3 The membrane filter containing coliform colony(ies) must be transferred from the total coliform medium to the surface of Nutrient Agar + MUG medium. Each sheen colony should be marked with a permanent marker on the lid. Also, the lid and the base should be marked with a line to realign the lid should it be removed. (A portion of the colony may be transferred with a needle to the total coliform verification test before transfer to Nutrient Agar + MUG or after the 4-hour incubation time. Another method is to swab the entire membrane filter surface with a sterile cotton swab after the 4-hour incubation time on Nutrient Agar + MUG medium, and transfer to a total coliform verification test.)

5.4.3.4 Inoculated medium must be incubated at 35°±0.5C° for four hours.

5.4.3.5 Check the fluorescence using an ultraviolet lamp (365-366 nm) with a 6-watt bulb in a darkened area. Any amount of fluorescence in a halo around a sheen colony should be considered positive for *E. coli*.

5.4.4 MF method for detecting enterococci/fecal streptococci in ground water

5.4.4.1 Media

5.4.4.1.1 For mE agar (SM 9230C) for the detection of enterococci: Prepare basal mE agar. Then autoclave and cool in a 44-46°C water bath. Dissolve 0.48 g nalidixic acid and 0.4 mL 10 N NaOH into 10 mL of reagent-grade distilled water and mix. Filter-sterilize the solution, and add 5.2 mL per liter of basal mE agar. For triphenyl tetrazolium chloride (TTC), add 0.25 g of TTC to 25 mL of reagent-grade water, and warm to dissolve. Filter-sterilize the solution, and add 15 mL per liter of basal mE agar. Final pH should be 7.1± 0.2.

5.4.4.1.2 For m-Enterococcus agar (SM 9230C) for the detection of fecal streptococci (not enterococci): Heat to dissolve ingredients, but do not autoclave. Dispense into sterile petri plates (9 X 50 mm) (about 4 mL), and allow to solidify. Final pH should be 7.2±0.2.

5.4.4.1.3 For mEI agar (EPA Method 1600) for the detection of enterococci: Add 0.75 g indoxyl-β-D-glucoside to 1L of basal mE agar, and proceed according to paragraph 5.4.4.1.1, except that the preparation of TTC is as follows: Add 0.1 g of TTC to 10 mL of reagent-grade distilled water, and warm to dissolve. Filter-sterilize the solution, and add 2 mL per liter of medium. Final pH should be 7.1±0.2.

5.4.4.2 After filtering a 100-mL sample, place membrane in a petri dish on one of the agar media listed above. Serial dilutions should not normally be necessary for detecting enterococci in ground water.

5.4.4.3 If m-Enterococcus agar is used, incubate inverted plate at 35°±0.5°C for 48 hours and, using magnification and a fluorescent lamp, count all light and dark red colonies as fecal streptococci.

5.4.4.4 If mE agar is used, incubate inverted plate for 48 hours at 41°±0.5°C, and then transfer filter to EIA medium. Incubate at 41°± 0.5°C for 20-30 minutes and, using magnification and a fluorescent lamp, examine the colonies. Pink to red colonies on mE agar with a black or reddish brown precipitate on the underside of filter on EIA indicates the presence of enterococci.

5.4.4.5 If mEI agar is used, incubate inverted plate for 24 hours at 41°±0.5°C. Using magnification and small fluorescent lamp, examine both the top and bottom of the plate for colonies with a blue halo. A colony with a blue halo, regardless of colony color, indicates presence of enterococci.

5.5 Heterotrophic Plate Count (for enumerating heterotrophic bacteria in drinking water)

5.5.1 The Pour Plate Method (*Standard Methods* 9215B) or the SimPlate Method must be used for determining compliance with 40 CFR 141.74(a)(1) (also listed in Appendix G) and should also be used for testing reagent grade water. For systems that have been granted a variance from the Total Coliform Rule's maximum contaminant level (see variance criteria in the preamble of FR 56:1556-1557, January 15, 1991), any method in *Standard Methods*, Section 9215, *Heterotrophic Plate Count*, may be used with R2A medium, for enumerating heterotrophic bacteria in drinking water.

5.5.2 Media

Method	Medium	Final pH
Pour Plate	Plate count agar, also known as tryptone glucose yeast agar	7.0 ± 0.2
Pour Plate	R2A agar	7.2 ± 0.2
Spread Plate	R2A agar	7.2 ± 0.2
Membrane Filter	R2A agar	7.2 ± 0.2
SimPlate	Multiple enzyme substrate	7.2 ± 0.2

5.5.3 (For Pour Plate Method) Melted agar must be tempered at 44°-46°C in waterbath before pouring. Melted agar should be held no longer than three hours. Sterile agar medium should not be melted more than once.

5.5.4 (For Spread Plate Method) 15 mL of R2A agar medium (or other medium) should be poured into a petri dish (100 x 15 mm or 90 x 15 mm) and allowed to solidify.

5.5.5 Refrigerated medium may be stored in bottles or in screw-capped tubes for up to six months, or in petri dishes for up to two weeks. Prepared petri dishes with R2A medium may be stored for up to one week.

5.5.6 For most potable water samples, countable plates can be obtained by plating 1.0 mL and/or 0.1 mL volumes of the undiluted sample (dilutions may not be necessary for SimPlate, which has a counting range up to 738/mL). At least duplicate plates per dilution should be used.

5.5.7 (For Pour Plate Method) The sample must be aseptically pipetted onto the bottom of a sterile petri dish. Then at least 10-12 mL mL of tempered melted (44°-46°C) agar must be added to each petri dish. The sample and melted agar must be mixed carefully to avoid spillage. After agar plates have solidified on a level surface, the plates must be inverted and incubated at 35°±0.5°C for 48±3 hours. Plates should be stacked no more than four high and arranged in the incubator to allow proper air circulation and to maintain uniform incubation temperature. Avoid excessive humidity in the incubator to reduce the possibility of spreader formation on the agar medium. Also avoid excessive drying of the agar medium; agar medium in plates should not lose more than 15% by weight during 48 hours of incubation.

5.5.8 (For Spread Plate Method) 0.1 or 0.5 mL of the sample (or dilution) should be pipetted onto the surface of the predried agar in the plate, and then spread over the entire surface of the agar using a sterile bent glass rod. The inoculum should be absorbed completely by the agar before the plate is inverted and incubated. The plate should be incubated at 20°-28°C for 5-7 days.

5.5.9 (For Membrane Filter Technique) The volume to be filtered should yield between 20-200 colonies. The filter is transferred to a petri dish containing 5 mL of solidified R2A medium, and incubated at 20°-28°C for 5-7 days. If plates with loose-fitting lids are used, plates should be placed in a plastic box with a close fitting lid containing moistened paper towels. Paper towels should be rewetted as necessary to maintain moisture. Colonies should be counted using a stereoscopic microscope at 10-15X amplification.

5.5.10 (For SimPlate Method)

5.5.10.1 Unit Dose (for a single sample). A10-mL volume of test sample is added to a test tube containing dehydrated SimPlate medium. Then the dissolved medium should be poured onto the center of a plate containing 84 small wells (provided by the manufacturer, IDEXX Laboratories, Inc.). Alternatively, 9 mL of sterile diluent (D.I. water, distilled water, or buffered water [*Standard Methods*, 9050 C,1a]) can be added to the tube, followed by 1-mL sample. Then follow the procedure as indicated above for the 10-mL sample. The mixture should be distributed evenly to the 84 wells on the plate, and the excess liquid drained into an absorbent pad on the plate. The plate should then be inverted (the fluid in each well is held in place by surface tension), and incubated for 45-72 hours at 35°±0.5°C. Bacterial density is determined by counting the number of wells that fluoresce under a 365-366 nm UV light, and converting this value to a Most Probable Number using the Unit Dose MPN table provided by the manufacturer. If 10-mL sample is used, read the Unit Dose MPN/mL directly. If a 1-mL sample is used, then correct the MPN/mL value by multiplying it by 10.

5.5.10.2 Multiple Dose (for 10 samples of 1 mL each): A 100-mL sterile diluent should be added to the dehydrated SimPlate medium to reconstitute, and shaken to dissolve. Then a 1.0-mL test sample should be pipetted to the center of a plate containing 84 small wells, followed by 9.0 mL of the reconstituted medium. Gently swirl plate to mix the sample and medium, and distribute the mixture evenly to the 84 wells on the plate. Then continue with the procedure indicated in paragraph 5.5.10.1 above, except that the Multi-Dose table supplied by the manufacturer should be used to determine the MPN/mL. If a dilution is made during sample preparation, then multiply the MPN/mL value by the dilution factor.

5.5.11 (For Pour Plate and Spread Plate Techniques) Colonies should be counted manually using a dark-field colony counter. In determining sample count, laboratories must only count plates having 30 to 300 colonies, except for plates inoculated with 1.0 mL of undiluted sample. Counts less than 30 for such plates are acceptable. (Fully automatic colony counters are not suitable because of the size and small number of colonies observed when potable water is analyzed for heterotrophic bacteria.)

QC 5.5.12 Each batch or flask of agar should be checked for sterility by pouring a final control plate. Data should be rejected if control is contaminated.

5.6 Coliphage (Draft Method 1601 and 1602, proposed Ground Water Rule)

Note: EPA Method 1601 and 1602 are performance-based methods for detecting the presence of male-specific (F^+) and somatic coliphage in ground water and other waters. (**Performance-based method**: In recognition of the variety of situations to which some methods may be applied, and in recognition of continuing technological advances, some methods are performance-based. A performance-based method permits laboratories to modify or omit steps or procedures, provided that all performance requirements set forth in the validated methods are met. Any steps that may not be modified or omitted must be specified in the method.)

5.6.1 EPA Method 1601: Male-specific (F^+) and Somatic Coliphage in Water by Two-Step Enrichment Procedure

Method Summary: A 100-mL (or 1-L water sample) is supplemented with magnesium chloride, log-phase host

bacteria (*E. coli* F_{amp} for male-specific coliphage and *E. coli* CN-13 for somatic coliphage), and Tryptic Soy Broth (TSB) as an enrichment step for coliphage. After an overnight incubation, samples are "spotted" onto a lawn of host bacteria specific for each type of coliphage, incubated, and examined for circular lysis zones, which indicate the presence of coliphage.

5.6.1.1 Media

5.6.1.1.1 Antibiotic stocks— Antibiotics must always be added to medium ***after*** the medium has been autoclaved. Store frozen at -20°C for up to one year. Thaw at room temperature or rapidly in a water bath up to 37°C and mix well prior to use. ***Please note: Antibiotics may be toxic. Wear suitable protective clothing, gloves, and eye/face protection and use in a chemical fume hood.***

5.6.1.1.2 10X Tryptic Soy Broth (TSB)—Store at 1°-5°C until use.

5.6.1.1.3 1.5% Tryptic Soy Agar (TSA)—If not used immediately after adding antibiotic and letting the plated medium solidify, store the plates inverted at 1°-5°C for up to 2 weeks.

5.6.1.1.4 0.7% TSA top agar tubes with appropriate antibiotics—Dispense 5 mL per sterile 10-mL tube, label, and keep at 45°- 48°C until use. Tubes must be used the day they are prepared.

5.6.1.1.5 Spot plates—Condensation may accumulate at the edges of stored spot plates and may drip over agar surface if tilted, ruining the spot pattern. If the stored spot plates have condensation, incubate plates for approximately 10 minutes to reduce condensation prior to inoculation. Spot plates may be used that day or stored at 1°-5°C for up to four days.

5.6.1.2 Coliphage stock

5.6.1.2.1 MS2 (ATCC#15597-B1, male-specific) and phi-X 174 stock coliphage (ATCC#13706-B1, somatic)—May be stored at 2-8°C for up to 5 years. Refer to http://www.atcc.org for initial preparation of pure coliphage stock.

5.6.1.2.2 Analysis of raw sewage filtrate should begin within 24 hours of collection.

5.6.1.2.3 Allow the raw sewage to settle at 1°-5°C for 1 to 3 hours. This will make the filtration process easier.

5.6.1.2.4 Hold the assembly over a 15-mL polypropylene tube with screw-cap or snap-cap, insert the plunger into the syringe barrel, and push the sewage through the filter into the sterile tube. If filter clogs, change it as necessary and continue to filter sewage until at least 10 mL of filtered sewage is obtained in the 15-mL polypropylene tube (filtration may require use of numerous filters).

5.6.1.2.5 If filtrate is stored more than 24 hours, it must be re-titered before use.

5.6.1.3 Host bacteria stock cultures

5.6.1.3.1 Frozen host bacteria stock cultures—After preparation, freeze host bacteria stock cultures at -70°C, if possible. Cultures can be frozen at -20°C if the laboratory does not have the capability to freeze samples at -70°C. Host bacteria stored at -70°C may be retained for up to one year. If stored at -20°C, the host bacteria may be retained for up to two months.

5.6.1.3.2 Overnight host bacteria stock cultures—After preparation, chill on wet ice or at 1°-5°C until ready for use.

5.6.1.3.3 Log-phase host bacteria stock cultures—After preparation, chill on wet ice or at 1°-5°C to slow replication until ready for use. The suspension may be stored up to 48 hours. However, the best results occur when cultures are used immediately (within 6 hours). Store remaining bacterial host culture at 1°-5°C overnight to inoculate flasks for the preparation of new log-phase bacterial hosts.

5.6.1.4 General QC

5.6.1.4.1 Initial demonstration of capability (IDC). *The laboratory must demonstrate the ability to generate acceptable performance with this method by performing an IDC test before analyzing any field samples.* The IDC test consists of ten reagent water samples spiked with enumerated sewage or equivalent at 1-2 PFU per sample for each coliphage type used, according to the IDC Table below. A minimum number of samples must be positive, depending on coliphage type used (see IDC Table). Spike samples in "bulk" at concentrations in the Table. Tests must be accompanied by a method blank for each coliphage type.

5.6.1.4.2 Method blanks. *The laboratory must analyze method blanks (reagent water sample containing no coliphage) to demonstrate freedom from contamination.* For each coliphage type used, prepare and analyze a sterile reagent water sample containing no coliphage using the same procedure used for analysis of the field or QC samples. At a minimum, the laboratory must analyze one method blank for each spot plate used for field samples. In an effort to determine if cross-contamination is an issue, the sterile method blank should be spotted onto the lawn of host bacteria immediately following the positive control spot.

5.6.1.4.3 Positive controls. *The laboratory must analyze positive controls to ensure that stock coliphage suspensions, host bacterial cultures, and growth media are performing properly.* For each coliphage type used, a 100-mL reagent water sample must be spiked with 20 PFU from sewage filtrate or 60 PFU from a pure coliphage stock culture. The laboratory must inoculate one positive control spot for each spot plate used for field samples. If multiple spot plates are inoculated with samples on the same day, a single enriched positive control sample may be used to inoculate multiple spot plates on that day.

5.6.1.4.4 Matrix spikes (MS). *To assess method performance in a given source water matrix, the laboratory must analyze one set of MS samples for each coliphage type when samples are first received from a ground water source for which the laboratory has never before analyzed samples.* For each coliphage type analyzed, three field samples are spiked with 1-2 PFU. At a minimum, one out of the three MS samples for each coliphage type must be positive for method performance to be considered acceptable for that ground water source. If the MS results are unacceptable, and the ODC sample and positive control sample results associated with this batch of samples are acceptable, a matrix interference may be causing the poor results. In addition, the laboratory must analyze one set of MS samples on an ongoing basis after every 20^{th} field sample for each ground water source. (For example, when a laboratory receives the first sample from a source, the laboratory must obtain additional aliquots of the field samples to be used for the MS test. When the laboratory receives the 20^{th} field sample from this site, additional aliquots of this sample must be collected and spiked.) MS samples should be collected at the same time as routine field samples. Spike samples in "bulk" at the concentrations indicated in the MS and ODC Table below.

5.6.1.4.5 Ongoing demonstration of capability (ODC). *The laboratory must demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples.* For each coliphage type used, three reagent water samples are spiked with 1-2 PFU. The ODC test samples are analyzed exactly like field samples, and at a minimum, one out of three ODC test samples must be positive for each coliphage type used. If not, method performance is unacceptable, and analysis of field samples must be stopped. Identify and correct the problem and demonstrate acceptable performance through analysis of another ODC test before continuing with the analysis of field samples. The laboratory must analyze one set of ODC samples after every 20 field and MS samples or one per week, whichever occurs more frequently. Spike samples in "bulk" at the concentrations indicated in the MS and ODC Table below.

5.6.1.4.6 Performance studies. The laboratory should periodically analyze an external QC sample, such as a performance testing sample, when available. The laboratory should also participate in available interlaboratory performance studies conducted by local, State, and federal agencies or commercial organizations. The laboratory should review results, correct unsatisfactory performance, and record corrective actions.

Initial demonstration of laboratory capability (IDC) for Method 1601

Coliphage type	Sample size[1]	Target spike concentration (PFU per sample)	"Bulk" volume to be spiked	Bulk spike concentration (PFU per bulk volume)	Minimum number of positive samples out of 10
F^+	100-mL	1.3	1000 mL	13	5
Somatic	100-mL	1.5	1000 mL	15	5

[1] A 100-mL sample is required under the Ground Water Rule. However, for other purposes, this test may be used with a 1-L sample volume. Because IDC samples should be analyzed just like field samples, including sample volumes, the IDC analyses should be performed at the 1-L sample volume when the laboratory is evaluating 1-L samples. (The IDC procedure for 1-L samples is provided in the protocol to Method 1601, Table 1.)

MS and ODC sample spiking requirements for ongoing evaluation of Method 1601 performance

Coliphage type	Sample size[1]	Target spike concentration (PFU per sample)	Number of samples that must be spiked (≥1 must be positive)	"Bulk" volume to be spiked	Bulk spike concentration (PFU per bulk volume)
F^+	100-mL	1.3	3	300-mL	3.9
Somatic	100-mL	1.5	3	300-mL	4.5

[1] A 100-mL sample is required under the Ground Water Rule. However, for other purposes, this test may be used with a 1-L sample volume. Because ODC and MS samples should be analyzed just like field samples, including sample volumes, the ODC and MS analyses should be performed at the 1-L sample volume when the laboratory is evaluating 1-L samples. (The MS and ODC procedure for 1-L samples is provided in the protocol to Method 1601, Tables 2.)

5.6.2 EPA Method 1602: Male-specific (F^+) and Somatic Coliphage in Water by Single Layer Agar (SAL) Procedure

Method Summary: Method 1602 is a performance-based method for detecting or enumerating male-specific (F^+) and somatic coliphage in ground water and other waters. A 100-mL ground water sample is assayed by adding magnesium chloride and host bacteria (*E. coli* F_{amp} for F^+ coliphage and *E. coli* CN-13 for somatic coliphage), and then adding the sample/host bacteria mixture to 100 mL of double-strength molten Tryptic Soy Agar containing the appropriate antibiotic. The sample is thoroughly mixed and the total volume is poured into 5 to 10 plates (dependent on plate size). After an overnight incubation, any circular lysis zones (plaques) indicate the presence of coliphage.

5.6.2.1. Media--Please refer to Section 5.6.1 for antibiotic stocks, 10X Tryptic Soy Broth (TSB), 1.5% Tryptic Soy Agar (TSA), 0.7% TSA top agar tubes with appropriate antibiotics, and spot plates.

5.6.2.1.1 Double Strength Tryptic Soy Agar (2X TSA)—Medium may become darker after autoclaving but this should not affect media performance.

5.6.2.1.2 2X TSA with appropriate antibiotics—Keep molten at 45°-48°C in water bath until use. Agar must be used the day of preparation.

5.6.2.2 Coliphage stock—Please refer to Section 5.6.1.2 for coliphage stock.

5.6.2.3 Host bacteria stock cultures — Please refer to Section 5.6.1.3 for host bacteria stock cultures.

5.6.2.4 General QC

5.6.2.4.1 Initial precision and recovery (IPR). *The laboratory must demonstrate the ability to perform this method acceptably by performing an IPR test before analyzing any field samples.* Four reagent water

samples for each coliphage type are required for the IPR test. IPR samples must be spiked in bulk to yield a target spike concentration of 80 PFU per sample. IPR samples must be spiked with enumerated sewage filtrate or equivalent. The relative standard deviation of the recovery (RSD_p) and the average percent recovery ($\bar{x}$) based on all four sample results for each coliphage type should meet the acceptance criteria in the QC acceptance table below.

5.6.2.4.2 Method blanks. *The laboratory must analyze method blanks (reagent water sample containing no coliphage) to demonstrate freedom from contamination.* The laboratory must analyze one method blank with each analytical batch. For each coliphage type used, prepare and analyze a sterile reagent water sample containing no coliphage using the same procedure as used for analysis of the field or QC samples. An analytical batch is defined as all samples analyzed during a single day, up to a maximum of 20 samples (field samples and matrix spike samples) per coliphage type.

5.6.2.4.3 Matrix spikes (MS). *To assess method performance in a given matrix, the laboratory must analyze one set of MS samples for each coliphage type when samples are first received from a ground water source for which the laboratory has never before analyzed samples.* The MS analysis is performed on an additional (second) sample aliquot collected from the ground water source at the same time as the routine field sample. If the laboratory routinely analyzes samples from one or more ground water sources, one MS analysis must be performed per 20 field samples. For example, when a laboratory receives the first sample from a source, the laboratory must obtain a second aliquot of this sample to be used for the MS. When the laboratory receives the 20th sample from this site, a separate aliquot of this 20th sample must be collected and spiked. Compare the coliphage recovery with the corresponding limits in the QC Table below. If the recovery for coliphage falls outside its limit, method performance is unacceptable for that sample. If the results for the OPR sample associated with this batch of samples are within their respective control limits, a matrix interference may be causing poor recovery. If the results for the OPR are not within their control limits, method performance is unacceptable (see Section 5.6.2.4.4). The problem should be identified and corrected, and the matrix spike and associated field sample(s) should be qualified. The recovery should be maintained on a control chart and updated on a regular basis.

5.6.2.4.4 Ongoing precision and recovery (OPR). *The laboratory must, on an ongoing basis, demonstrate acceptable performance through analysis of an OPR sample.* For each coliphage type used, a reagent water sample is spiked with approximately 80 PFU. The OPR is analyzed exactly like a field sample. The laboratory must analyze one OPR sample for each analytical batch. An analytical batch is defined as all samples analyzed during a single day, up to a maximum of 20 samples (field samples and matrix spike samples) per coliphage type used. ***Please note: the OPR serves as the positive control for Method 1602.*** Compare the OPR percent recovery (R) with the corresponding limits for ongoing precision and recovery in the QC Table below. If R meets the acceptance criteria, system performance is acceptable and analysis of samples may continue. If R falls outside the range for recovery, method performance is unacceptable, and analysis of field samples must be stopped. Identify and correct the problem and demonstrate acceptable performance through successful analysis of another OPR test before continuing with the analysis of field samples.

5.6.2.4.5 Performance studies. The laboratory should periodically analyze an external QC sample, such as a performance testing sample, when available. The laboratory also should participate in available interlaboratory performance studies conducted by local, state, and federal agencies or commercial organizations. The laboratory should review results, correct unsatisfactory performance, and record corrective actions.

Quality control acceptance criteria for Method 1602

Performance test	Male-specific acceptance criteria	Somatic acceptance criteria
Initial precision and recovery (IPR)		
• Mean percent recovery	9% - 130%	86% - 177%
• Precision (as maximum relative standard deviation)	46%	23%
Ongoing precision and recovery (OPR) as percent recovery	4% - 135%	79% - 183%
Matrix spike (MS)		
• MS percent recovery	Detect - 120%	48% - 291%
Matrix spike, matrix spike duplicate (MS/MSD)		
• Mean percent recovery for MS/MSD	Detect - 120%	48% - 291%
• Precision (as maximum relative percent difference of MS/MSD)	57%	28%

6. Sample Collection, Handling, and Preservation

Paragraphs 6.1-6.5 are applicable to those laboratories that collect samples. However, all laboratories should make an effort to ensure proper sample collection; all laboratories are responsible for paragraph 6.6.

6.1 Sample Collector

The sample collector should be trained in aseptic sampling procedures and, if required, approved by the appropriate regulatory authority or its designated representative.

6.2 Sampling

6.2.1 (For TCR) Samples must be representative of the water distribution system. Water taps used for sampling should be free of aerators, strainers, hose attachments, mixing type faucets, and purification devices. Cold water taps should be used. The service line must be cleared before sampling by maintaining a steady water flow for at least two minutes (until a steady water temperature is achieved). At least 100 mL of sample must be collected, allowing at least a 1-inch air space to facilitate mixing of the sample by shaking. Immediately after collection, a sample information form should be completed (see paragraph 6.5). See Section 3.15.4 regarding sample dechlorination. If a sample bottle is filled too full to allow for proper mixing, do not pour off and discard a portion of the sample. Rather, pour the entire sample into a larger sterile container, mix properly, and proceed with the analysis.

6.2.2 (For SWTR) Source water samples must be representative of the source of supply, collected not too far from the point of intake, but at a reasonable distance from the bank or shore. The sample volume should be sufficient to perform all the tests required.

6.2.3 (For coliphage analysis under GWR) A 100-mL sample volume is required for the assay. Collection of an additional 100-mL water sample would allow for sample re-analysis, if necessary (e.g., if the positive or negative controls fail). To ensure sufficient sample volume, an additional 50-mL water sample should be collected.

6.2.4 (For *E. coli* and enterococci under GWR) A 100-mL sample volume is required for the assay.

6.3 Sample Icing

6.3.1 (Bacterial samples) Samplers are encouraged, but not required, to hold drinking water samples at <10°C during transit to the laboratory. Source water samples required by the Surface Water Treatment Rule (SWTR) must be held

at <10°C during transit (see *Standard Methods*, Section 9060B). Laboratories should reject samples that have been frozen.

6.3.2 (For coliphage analysis under GWR) Ship samples at <10°C using wet ice, Blue Ice®, or similar products to maintain temperature, and store at 1°-5°C. Samples should not be frozen.

QC **6.3.3** For SWTR samples and coliphage samples, sample temperature upon receipt should be recorded. A sample that has a temperature upon receipt of >10°C , whether iced or not, should be flagged unless the time since sample collection has been less than two hours.

6.4 Sample Holding/Travel Time

6.4.1 For the analysis of total coliforms in drinking water, the time between sample collection and the placement of sample in the incubator must not exceed 30 hours (per regulation at 40 CFR 141.21(f)(3)). All samples received in the laboratory should be analyzed on the day of receipt. If the laboratory receives the sample late in the day, the sample may be refrigerated overnight as long as analysis begins within 30 hours of sample collection.

6.4.2 The time from sample collection to placement of the sample in the incubator for total coliforms and fecal coliforms in surface water sources, and heterotrophic bacteria in drinking water, must not exceed eight hours (per regulation at 40 CFR 141.74(a)(1)).

6.4.3 (For coliphage analysis) The time between sample collection and the placement of sample in the incubator must not exceed 48 hours. The time from sewage sample collection to analysis of QC spiking suspensions may not exceed 24 hours, unless re-titered and titer has not decreased by more than 50%. If titer has not decreased by more than 50%, the sample can be stored for up to 72 hours.

6.4.4 (For *E. coli* and enterococci under GWR) The time between sample collection and the placement of sample in the incubator must not exceed 30 hours.

6.5 Sample Information Form

After collection, the sampler should enter on a sample information form, in indelible ink, the following information:

- Name of system (public water system site identification number, if available)
- Sample identification (if any)
- Sample site location
- Sample type (e.g., routine distribution system sample, repeat sample, raw or process water, other special purpose sample)
- Date and time of collection
- Analysis requested
- Disinfectant residual
- Name of sampler
- Any remarks

6.6 Chain-of-Custody

Sample collectors and laboratories must follow applicable State regulations pertaining to chain-of-custody. An example of such a plan is provided in Appendix A.

7. Quality Assurance

7.1 A written QA plan should be prepared and followed (see Chapter III). The QA plan should be available for inspection by the certification officer. As specified by the QA plan, a laboratory that performs its own calibration of equipment or supplies (e.g., thermometers) should have a Standard Operating Procedure available for review. If a laboratory wishes to perform additional QA beyond those in this manual, the laboratory may refer to *Standard Methods*, Section 9020, *Quality Assurance (Quality Assurance/Quality Control*, in 20th ed.).

7.2 States are encouraged to establish proficiency testing (PT) as part of their drinking water certification program for microbiology. A laboratory should successfully analyze at least one set of PT samples once every 12 months, for each method for which it is certified.

For methods used to test the presence or absence of an organism in a sample, each PT set should contain ten samples, all shipped at the same time in either a lyophilized, dehydrated, or aqueous state. The set should include samples, in various combinations, that contain total coliforms, fecal coliforms, *E. coli*, non-coliforms, and at least one blank. Each set should be used only with a single analytical method. To be acceptable, a laboratory should correctly analyze a minimum of nine of the ten samples, with no false-negative result (i.e., a single false-positive result may be acceptable).

Because even methods based upon the same principle (e.g., membrane filtration) may be quite dissimilar, a Region or State should consider certifying a laboratory only for those specific methods for which the laboratory has successfully analyzed a set of PT samples. The Table below reflects this approach, and identifies the few methods that may be sufficiently similar to allow a laboratory to be certified for more than one method upon successful completion of a single set of PT samples.

Method Category	Specific Method[1]
Fermentation broth method	LTB or P-A broth, followed by BGLB and either EC or EC-MUG
Fermentation broth method	A-1 broth (fecal coliform, SWTR only)
Enzyme substrate method	Colilert or Colilert 18
Enzyme substrate method	Colisure
Enzyme substrate method	Readycult or Fluorocult LMX
Enzyme substrate method	E*Colite
Enzyme substrate method	Colitag
Membrane filter method	M-Endo or LES Endo, followed by BGLB and either EC, EC-MUG, or NA-MUG
Membrane filter method	MI Medium
Membrane filter method	Coliscan
Membrane filter method	m-ColiBlue24
Membrane filter method	Chromocult
Membrane filter method	mFC agar (fecal coliform, SWTR only)
HPC method	PCA
HPC method	SimPlate

[1] Separate set of proficiency test samples recommended for each cell. A single set of PT samples would cover every method within the same cell.

8. Records and Data Reporting

8.1 Legal Defensibility: Compliance monitoring data should be made legally defensible by keeping thorough and accurate records. The QA plan and/or SOPs should describe the policies and procedures used by the facility for record retention and storage. If samples are expected to become part of a legal action, chain-of-custody procedures should be used (See Appendix A).

8.2 Maintenance of Records: Public water systems are required to maintain records of microbiological analyses of compliance samples for five years (40 CFR 141.33). The laboratory should maintain easily accessible records for five years or until the next certification data audit is complete, whichever is longer. A change in ownership, merger, or closure of a laboratory does not cancel this requirement. The client water system should be notified before disposing of records so they may request copies if needed. This includes all raw data, calculations, and quality control data. These data files may be either hard copy, microfiche or electronic. Electronic data should always be backed up by protected tape or disk or hard

copy. If the laboratory changes its computer hardware or software, it should make provisions for transferring old data to the new system so that it remains retrievable within the time frames specified above. Data which is expected to become part of a legal action will probably need to be maintained for a longer period of time. Check with your legal counsel. See *Good Automated Laboratory Practices*, EPA 2185, Office of Information Management, Research Triangle Park, NC 27711, 8/10/95.

8.3 Sampling Records: Data should be recorded in ink with any changes lined through such that original entry is visible. Changes should be initialed and dated. The following information should be readily available in a summary or other record(s):

8.3.1 Sample information form, from 6.5 above
8.3.2 Date and time of sample receipt by the laboratory
8.3.3 Name of laboratory person receiving the sample
8.3.4 Any deficiency in the condition of the sample. A sample should be invalidated for the following reasons:
- Time between sample collection and receipt by laboratory has been exceeded
- Presence of disinfectant in sample is noticed (e.g., odor)
- Evidence of freezing
- Use of a container not approved by the laboratory for the purpose intended
- Insufficient sample volume (e.g., <100 mL)
- Presence of interfering contaminant, if noticed (e.g., hydrocarbons, cleansers, heavy metals, etc.)
- Sample temperature exceeds the maximum allowable

8.4 ***Analytical Records***: Data should be recorded in ink with any changes lined through such that original entry is visible. Changes should be initialed and dated. The following information should be readily available in a summary or other record(s):

8.4.1 Laboratory sample identification
8.4.2 Date and time analysis begins
8.4.3 Laboratory and a signature or initials of person(s) performing analysis
8.4.4 Analytical technique or method used
8.4.5 All items marked QC
8.4.6 Results of analyses

8.5 Preventive Maintenance
Laboratories should maintain preventive maintenance and repair activities records for all instruments and equipment (including pH meters, analytical balances, incubators, refrigerators, autoclaves, and water baths). Records should be kept for five years in a manner that allows for easy inspection.

9. Action Response to Laboratory Results

9.1 Testing Total Coliform-Positive Cultures
For the Total Coliform Rule, laboratories must test all total coliform-positive cultures for the presence of either fecal coliforms or *E. coli*.

9.2 Notification of Positive Results

9.2.1 For the Total Coliform Rule, laboratories must promptly notify the proper authority of a positive total coliform, fecal coliform, or *E. coli* result, so that appropriate follow-up actions (e.g., collection of repeat samples) can be conducted (see 40 CFR 141.21(b) and (e), and 141.31, etc.).

9.2.2 If any sample is fecal coliform- or *E. coli*-positive, "the system must notify the State by the end of the day when the system is notified of the test result, unless the system is notified of the result after the State office is closed, in which case the system must notify the State before the end of the next business day." (40 CFR 141.21(e)(1)).

9.2.3 A total coliform-positive result is based on the confirmed phase if the Multiple Tube Fermentation Technique or Presence-Absence (P-A) Coliform Test is used, or the verified test for the Membrane Filter Technique if M-Endo medium or LES Endo agar is used. No requirement exists to confirm a total coliform-positive result using Colilert, Colisure, MI agar, E*Colite, MI agar, m-ColiBlue24, Chromocult, Readycult/Fluorocult, Coliscan, or Colitag test. Also, no requirement exists to confirm a positive fecal coliform or *E. coli* test. In those rare cases where a presumptive total coliform-positive culture does not confirm/verify as such, but is found to be fecal coliform or *E. coli*-positive, the sample is considered total coliform-positive and fecal coliform/*E. coli*-positive.

9.3 Notification of Total Coliform Interference

For the Total Coliform Rule, the laboratory must promptly notify the proper authority (usually the water system) when results indicate that non-coliforms may have interfered with the total coliform analysis, as described in 40 CFR 141.21(c)(2).

Example Checklists for On-site Evaluation of Laboratories Analyzing Drinking Water

MICRO CHECKLISTS

General Audit Information

Laboratory:
Mailing Address (mailing address of owner if different):
Street
City, State, Zip code
Audit Location (if different):
Telephone:
Fax:
E-mail:
Other:
Audit Organization:
Auditors/Signatures:
Audit Date(s):

Laboratory Personnel

Position/Title	Name	Education Level Degree/Major	Specialized Training	Present Specialty	Experience, including # yrs at current position
Laboratory Supervisor					
Laboratory Consultant					
Primary Analyst					
Analyst 2					
Analyst 3					
Analyst 4					
Others					